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TITLE: **ROLE OF MACROPHAGE-INDUCED INFLAMMATION IN MESOTHELIOMA**

PRINCIPAL INVESTIGATOR: **V. Courtney Broaddus, MD**

CONTRACTING ORGANIZATION:

The University of California

San Francisco, CA 94143-0962

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14. ABSTRACT: Macrophages are the first inflammatory cells to respond to asbestos fibers and we have shown that they are the predominant inflammatory cell in the asbestos-induced tumor, mesothelioma. We are beginning to unravel the role of the macrophage in this intractable tumor, using in vitro models of macrophages grown with mesothelioma cells, ex vivo models of human mesothelioma maintained as tumor fragment spheroids, and in 2 in vivo models of immunocompetent murine mesothelioma. In this first year, we have confirmed by immunohistochemistry and by flow cytometry of fresh tumor that macrophages are the main inflammatory cell in mesothelioma. We have shown that we can polarize macrophages to either an M1 (anti-tumor) or M2 (pro-tumor) phenotype and that M1-polarized macrophages (derived from a variety of sources including human mesothelioma) increase mesothelioma cell apoptosis and chemosensitivity. In our first study using an orthotopic murine mesothelioma model, the depletion of macrophages increased the mesothelioma response to chemotherapy. We aim to understand the role of the macrophage and how polarization or depletion of macrophages within the tumor can sensitize the mesothelioma cell to treatment.					
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I. INTRODUCTION

Inflammation is an early and consistent feature of mesothelioma [1, 2], an incurable tumor with a high level of resistance to chemotherapy [3]. Within this inflammatory milieu, we have discovered that there is a large population of macrophages, in a higher percentage than we have found in other thoracic malignancies. The macrophage is perhaps the earliest cell recruited to the asbestos fibers that are the cause of mesothelioma. After the tumor forms, macrophages are also, as we have found, a constant feature of mesothelioma. The association of macrophages appears thus to be a feature of mesothelioma during its initiation as well as its progression over four decades.

Macrophages, often known as tumor-associated macrophages, are found, albeit to a lesser extent, throughout many solid tumors. Increasingly, they are recognized as a key factor enhancing the malignant features of the tumor [4]. In separate tumors, a greater extent of macrophage infiltration in a tumor correlates with worse prognosis [5, 6]. Macrophages are now understood to have different phenotypes, directed by their cellular and cytokine environment [7]. The classic macrophage phenotype, termed M1 in analogy to the Th1 phenotype of lymphocytes, is directed to phagocytosis and anti-bacterial actions. This classic activation is seen in the pro-inflammatory environment, where LPS or interferons are prominent. More recently, an M2 phenotype was described that is directed by other cytokines, namely IL4, TGF beta. This alternatively activated macrophage is considered to be important at for the resolution of inflammation, dampening the inflammatory response and orchestrating a healing or tissue remodeling phase [8].

We wish to understand the mechanisms by which macrophages influence mesothelioma cells and whether we can manipulate the tumor-associated macrophages within the tumor by deletion or by alteration of the macrophage phenotype to sensitize tumors to apoptosis. To address these questions, we have several in vitro, ex vivo and in vivo approaches to investigate the mechanisms of macrophage-mesothelioma cell interactions. Our studies are designed to test the hypothesis that macrophages influence mesothelioma cell survival and can be manipulated to enhance mesothelioma cell apoptosis and response to chemotherapy.

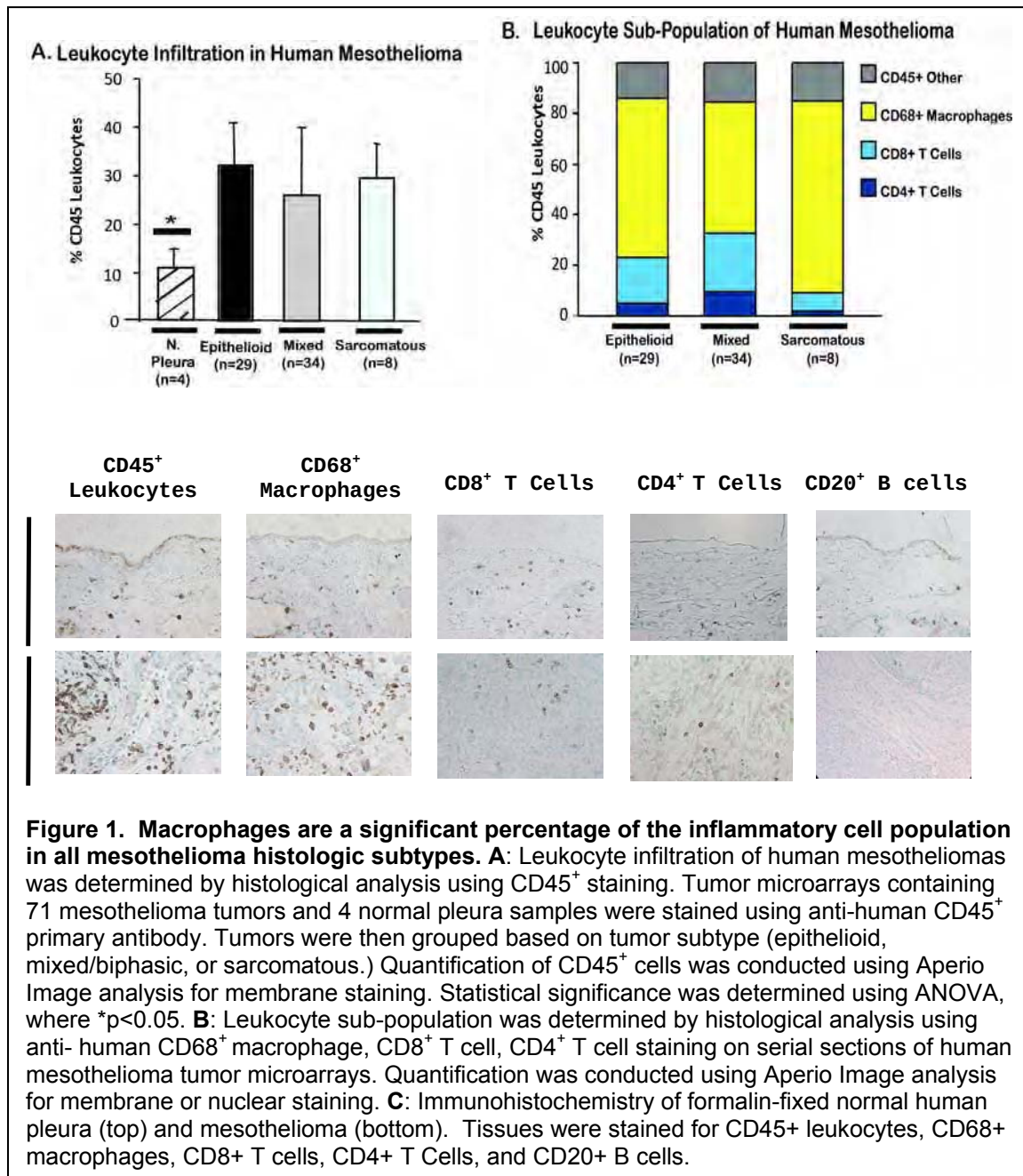
II. RESEARCH ACCOMPLISHMENTS

Task 1. To determine the functional significance of macrophage phenotype on mesothelioma cell survival.

a. Elucidate the percentage of immune cells (CD45+) in human mesothelioma tumors and correlate immune cell infiltration with histopathologic subtype (months 1-6).

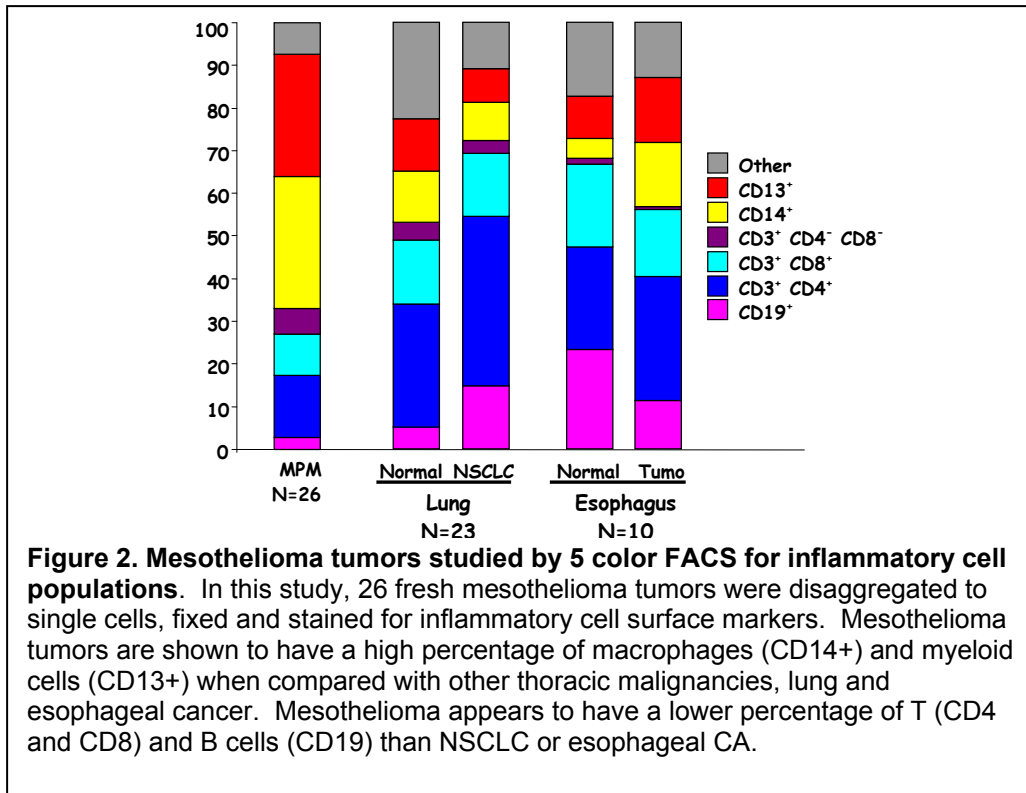
Using tumor tissue microarrays of 71 mesothelioma tumors in the laboratory including new tumors obtained over the first year of funding, we have performed extensive immunohistochemical staining for immune cell populations and have correlated the macrophage population with the histologic subtype of mesothelioma. The intensity of staining of the cell population was assessed by digital analysis software (Aperio) to avoid investigator bias. The tumors were from patients who had not received chemotherapy;

interestingly, in other studies, there were no differences noted in those tumors from patients given prior chemotherapy. In mesothelioma, as in other tumors studied in the laboratory, inflammatory cells (CD45) make up a large percentage of the cells within the tumor (**Figure 1A**). This percentage is similar in each mesothelioma pathologic subtype. The macrophages are a large percentage of the inflammatory cell population (**Figure 1B**). It is also interesting that, in the few sarcomatous tumors studied so far (n=8), macrophages may constitute a higher percentage of the total inflammatory cell population than in epithelial or mixed tumors. If indeed macrophages act to support tumor survival, then a high percentage of macrophages in sarcomatous tumors could help explain the poor prognosis of this cell type. On the other hand, the ability to repolarize such macrophages might provide a useful therapeutic tool against such a recalcitrant histologic subtype of mesothelioma.

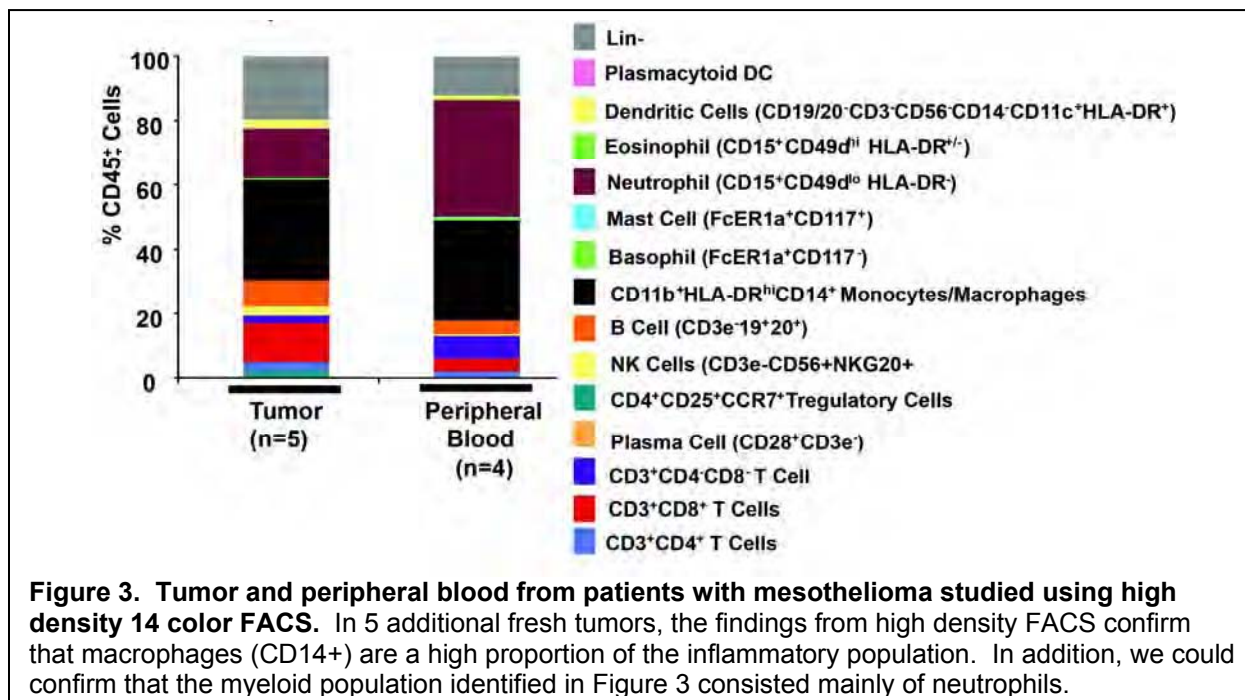


b. Determine the macrophage population (CD14+) as a percentage of the total immune cell population by flow cytometry (months 1-36).

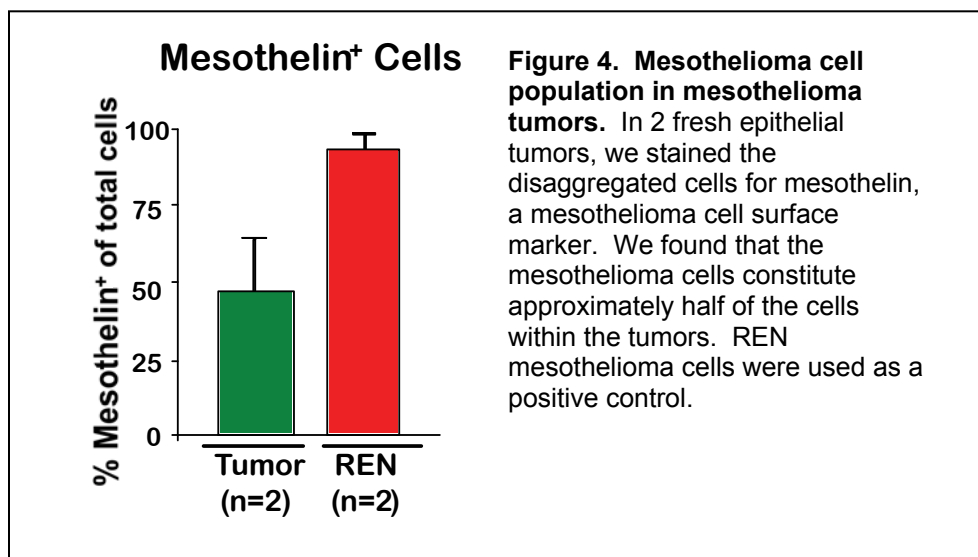
We have now analyzed 26 fresh tumors sent from our collaborators at the Brigham & Womens Hospital and from Dr. Jablons by FACS for inflammatory cell populations. Flow studies confirmed and also amplified the results from the immunohistochemical studies (described in Task 1a). Initially we analysed tumors using a 5 color FACS and were thus able to determine the major inflammatory cell populations (**Figure 2**); within the last 4 months, we acquired a high density FACS, able to detect 14 colors and thus to refine the analysis for many inflammatory subtypes (**Figure 3**).



Using high density FACS, we have now studied 5 fresh tumors sent from Boston along with 4 peripheral blood specimens from patients. These high density studies have confirmed the high percentage of macrophages in these tumors (approximately 30% of CD45+ cells). Thus, by immunohistochemistry and flow studies (5 color and 14 color), macrophages represent the predominant inflammatory cell within the inflammatory cell population of mesothelioma tumors.

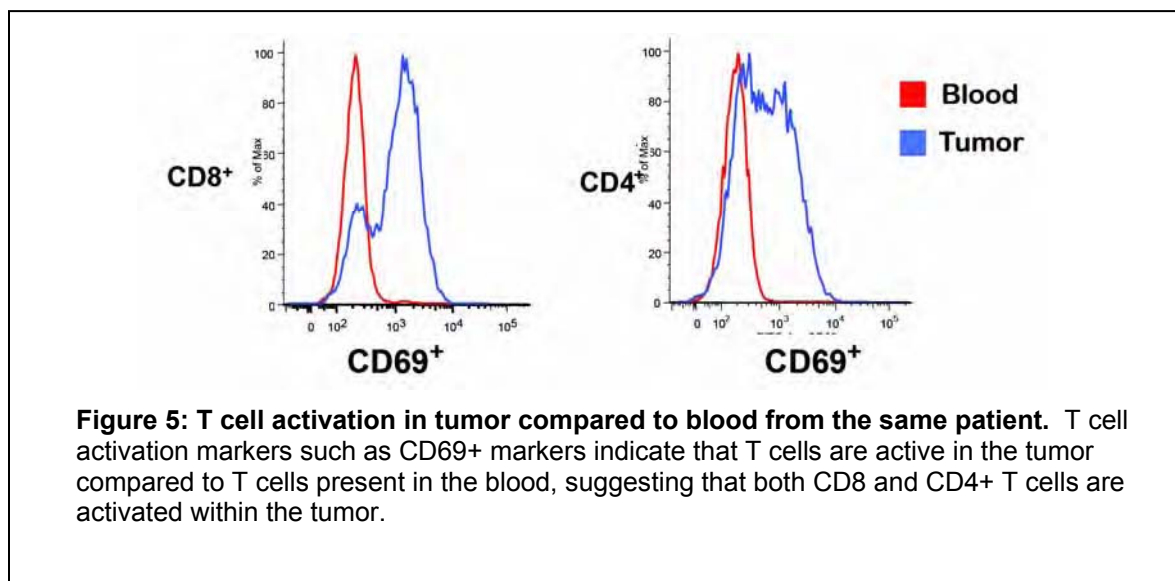


In additional FACS studies, we disaggregated the tumor and stained cells for mesothelin to determine the percentage of cells within tumors that were mesothelioma cells (mesothelin-positive) (**Figure 4**). These results will be of value for future studies in which mesothelin-positive cells will be sorted and used in hybrid spheroid studies. We find that the actual malignant cells constitute approximately 50% of the tumor, which is consistent with the finding that the inflammatory cell population constitutes approximately 30% of the tumor (see **Figure 1A**). The additional 20% of the cell population may consist of non-malignant, non-inflammatory tumor-associated cells such as endothelial cells and fibroblasts.



c. Determine the profile of other immune cells within the microenvironment of the mesothelioma tumor using a panel of cell surface markers (months 1-36).

We are continuing to study the profile of the other immune cells using the high density FACS, as described above. This new FACS approach will enable the assessment of activation status within many of the inflammatory cell populations. As an example, we are using the CD69+ activation marker for T cells (CD4 and CD8) to compare the activation status of these T cells in tumor compared to blood from the same patients. In one tumor-blood comparison, the T cells were found to be activated within the tumor (**Figure 5**). The CD69 early activation marker has been reported to be activated in lymphocytes within tumors, leading to proliferation but not necessarily to a cytotoxic effector function. Nonetheless, the presence of T cells with early activation markers indicates the potential for such T cells to be stimulated to a cytotoxic effector function.



d. Confirm macrophage percentages by immunodetection of the same mesothelioma in fixed tissues (CD68+) (months 1-36).

In these ongoing studies, the percentage of macrophages using FACS is being compared to that found by immunohistochemistry. On average, using both immunohistochemistry and flow cytometry, macrophages constitute between 30-70% of the CD45+ (inflammatory) cell population in mesothelioma (**Figures 1B, 2, 3**). We have also found no significant difference in macrophage percentage in the same tumor when measured by immunohistochemistry on freshly fixed tissue and when measured 24 h later by flow cytometry.

e. Isolate macrophages from human mesothelioma disaggregated into single cells by flow cytometry for use in co-culture spheroids with mesothelioma cells (1-12).

Human macrophages were isolated successfully with high viability (80-90%) using Ficoll separation to remove the non-monocytic cells and then isolating CD14+ cells by bead isolation. These were placed on plastic to adhere and then differentiated using MCSF and 1% FBS for 2 days. Then, the macrophages were polarized with M1 or M2 cytokines (or M0= no cytokines) over 24 h, and they were lightly trypsinized and used to

prepare spheroids with human mesothelioma cell lines. The spheroids were exposed to polarizing cytokines for another 24 h and then treated with TRAIL plus gemcitabine for 24 h. The presence of human macrophages isolated from mesothelioma increased mesothelioma cell apoptotic response to treatment, especially when the macrophages were polarized toward an M1 phenotype (shown in Task 2g below).

f. Determine macrophage functional properties in mesothelioma using fixed tissues, by tissue microarray, and by immunohistochemistry for protein expression to define their M1 or M2 microenvironmental status (months 1-12)

Cytokine staining in tissue has proved to be a challenging technical problem and new approaches are being perfected for this and other studies. Fortunately, the fixed tissues are stored and the tumor tissue microarrays generated for these studies so the task is only postponed until the second year of funding. These tumors will serve as a tissue bank for this task and others for the next two years of the project.

g. Determine the M1 or M2 gene expression signature of macrophages by commercial global chip assay for RNA from tumor tissue or cultures of cells/spheroids for gene profile (months 1-12).

The quantitative PCR analysis of 7 mesothelioma tumors compared to normal tissues has shown an upregulation of message for several inflammatory cytokines, including CCL3, a key macrophage-produced inflammatory chemokine, and CSF-1, a macrophage stimulating and recruitment factor (**Figure 6**). Interestingly, in these first 7 tissues, we do not yet see a clear polarization signal. The elevation of IL10 is indicative of an M2 phenotype while the increase in IFN gamma would suggest an M1 phenotype. We continue to pursue these studies with additional fresh frozen mesothelioma samples; currently, we have 18 more frozen and ready to analyze. With additional data, including the protein expression data obtained as part of 2f, we expect a clearer picture to emerge. If a clear picture does not emerge, we will plan to disaggregate the mesothelioma cells from the tumor to study them away from the microenvironment. The isolated mesothelioma cells could then be studied for their message for cytokines and studied for their intracellular cytokine concentrations.

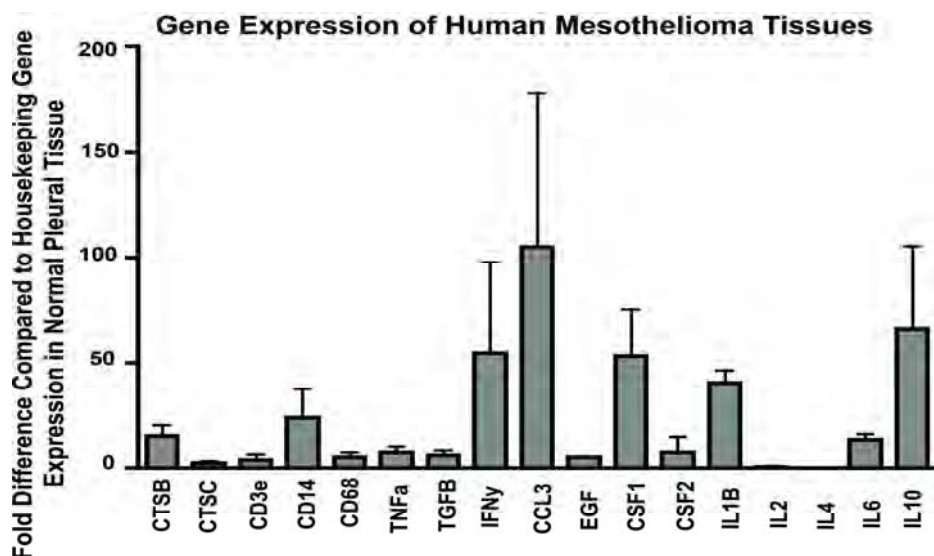


Figure 6. Quantitative PCR of Human Mesothelioma Tumors. 7 snap frozen human mesothelioma tumors were analyzed using quantitative PCR to determine gene expression of key genes indicating macrophage polarization. Mesothelioma tumor samples were run alongside normal pleural samples to measure change in gene expression compared to tumor-free patients. There is a significant increase in CD14, IFN- γ , IL-10 and CCL3 genes, indicating an inflammatory response. The pattern does not suggest a specific polarization state.

h. Analyze cytokines produced by mesothelioma tumor fragments by commercial cytokine bead assay from human mesothelioma grown as tumor fragments (months 1-12).

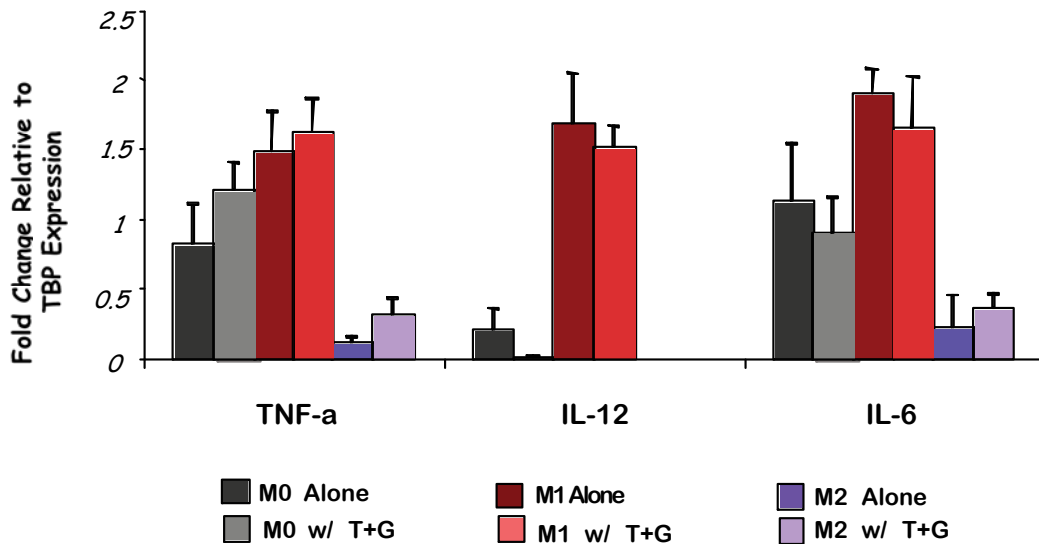
We have not had success in measuring cytokines from the tumors, despite use of several different assays. We believe the cytokines have been too dilute for consistent results and efforts to concentrate the samples are underway to allow measurement. At the same time, we are taking a different tack, to measure intracellular cytokines via flow cytometry. The protocol is now being perfected in the laboratory and will be applied to mesothelioma tumor tissue. There are several potential advantages of the intracellular cytokine assay compared to an ELISA which measures secreted, soluble cytokines. For one, the dilution of cytokines is not a factor. For another, we can co-stain the cells with an anti-mesothelin or an anti-CD14 antibody to determine what cytokines are produced specifically by the mesothelioma cells or macrophages, respectively. Thus, we have modified our plan for these studies and we expect them to be complete in the next funding cycle.

i. Analyze cytokines produced by multicellular spheroids made from either mesothelioma cells alone or mesothelioma cells plus macrophages (THP-differentiated) (months 1-12).

We have completed studies with hybrid spheroids showing that we are able to polarize the mesothelioma + macrophage hybrid spheroids to an M1 or M2 phenotype (**Figure 7**). In addition, we find that the apoptotic treatment using TRAIL or gemcitabine does not

alter the polarization phenotype of the hybrid spheroids. In these studies, we have also begun a more detailed study of M2 polarization, to M2a and M2c, which are induced by exposure to IL-4 and IL-10 respectively.

M1 “markers”



M2 “markers”

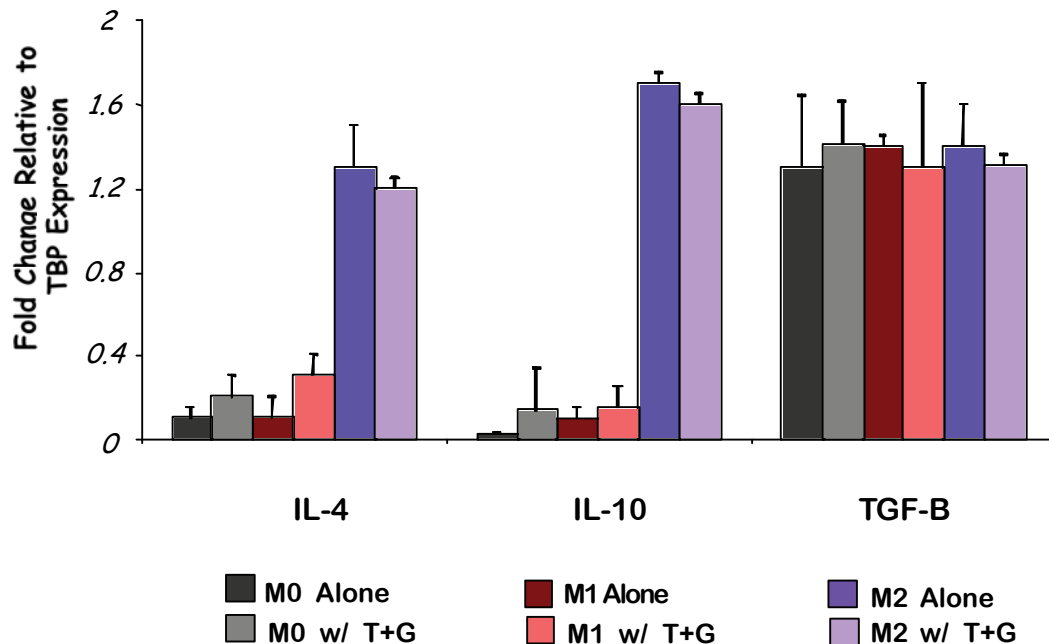


Figure 7. Cytokine Expression in Hybrid M28 Macrophages is Altered by Programming to an M1 or M2 Phenotype. Macrophages differentiated from peripheral blood were reprogrammed to either an M1 (IFN γ /LPS), M2a (IL4), or M2c (IL10) phenotype over 48 hours in culture. Reprogrammed macrophages were then incorporated with M28 human mesothelioma cells in poly-HEMA coated 96-well plates to form 3D hybrid spheroids. These hybrid spheroids were

grown in DMEM with 1% FBS with any additional polarizing cytokines (M1: IFN- γ /LPS, M2a: IL4; M2c: IL-10) for 24 hours. 18-20 spheroids were then harvested and fixed using RLT buffer. Spheroids were then harvested and fixed using RLT buffer. Samples were processed for RNA and cDNA synthesis. Real Time PCR was used to quantify gene expression for M1 Markers: TNF- α , IL-12 p40, and IL-6 as well as M2 markers: IL-4, IL-10, and TGF- β . Expression values were normalized against the expression of housekeeping genes, specifically, TBP.

j. Analyze changes in cytokine expression when spheroids (mesothelioma only or mesothelioma plus macrophage) are treated with TRAIL or TRAIL plus gemcitabine (months 1-12).

See above. There was no significant change in the polarization state or cytokine profile after treatment with TRAIL plus gemcitabine (T+G).

Task 2. To determine the functional significance of macrophages as regulators of mesothelioma apoptosis in vitro.

a. Confirm ability of clodronate-liposomes to deplete macrophages from multicellular spheroids made either with THP-1 differentiated macrophage cells or with primary mesothelioma-derived macrophages and determine optimal dose and timing (months 9-14).

In order to advance the in vivo projects, we used the clodronate more extensively in the in vivo model applying the dosing and timing schedule published for the in vivo setting. The study was readily performed in mice with GFP-labeled macrophages showing that the clodronate did indeed deplete all GFP-labeled cells from the peritoneal lavage in clodronate-treated mice. The studies are now being carried out in hybrid spheroids to establish the optimal dosing for the in vitro setting and should be completed as planned within the next 2 months.

b. Confirm ability of clodronate-liposomes to deplete macrophages from mesothelioma tumor fragment spheroids with dose and timing established above (months 12-24).

As above, we will begin these studies as planned this year, following the dose established as effective in the hybrid spheroids above (Task 2a).

c. Analyze effect of macrophages and of macrophage depletion on apoptosis of mesothelioma cells to treatment with TRAIL or TRAIL plus gemcitabine using multicellular spheroids either with no macrophages, macrophage-depleted or with macrophages (months 12-24).

We have begun these studies using the no macrophage and with macrophage hybrids, the macrophage-depleted studies will begin this year and will be important to show whether the clodronate itself alters the results. Growing the macrophages with the mesothelioma cells leads to a well formed chimeric structure we call a hybrid spheroid, with the macrophages well distributed throughout the spheroid (**Figure 8A**). We have extensively confirmed our preliminary studies that polarization of the macrophages to an M1 phenotype enhances the apoptosis of mesothelioma cells grown with them (**Figure 8B**). The macrophages that are not polarized (called M0) do not have a significant effect in these in vitro experiments. Polarization of the macrophages to an M1 phenotype however induces a robust pro-apoptotic effect. This effect, as shown in Figure 8 and reproduced in all our experiments, requires the presence of the macrophages; the polarization program has no effect on mesothelioma cells themselves. The pro-apoptotic activity of the macrophages in hybrid spheroids has now been reproduced in more than one cell line (M28 and REN), with at least 4 different sources of macrophages (derived from the THP-monocyte-like cell line, from peripheral blood monocytes, from banked blood monocytes, or from human mesothelioma tissue) and with different apoptotic treatments. We are confident of these findings and have worked to establish the optimal conditions. For example, we have found that freshly obtained macrophages, derived from peripheral blood monocytes, are more effective and active in the ability to induce apoptosis than those derived from banked blood. In certain cases, we have also been able to show that an M2 phenotype suppresses the mesothelioma cell phenotype; this result appears to be more dependent on the culture conditions, requiring a reduction in the background serum stimulation of the cells to 1-2% serum.

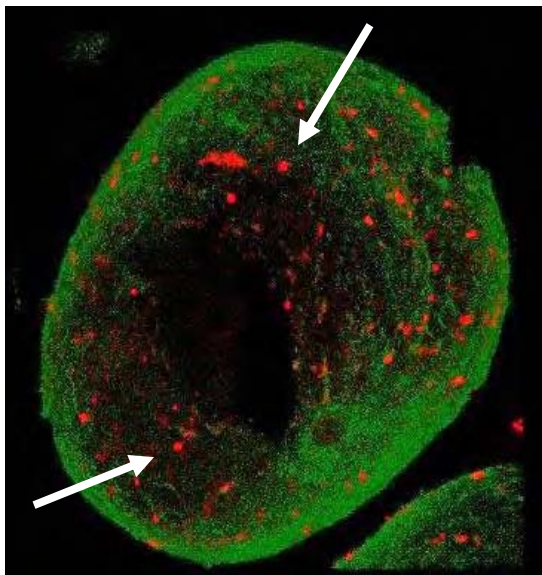


Figure 8A. Macrophages and mesothelioma cells form hybrid spheroids. This spheroid contains 10,000 mesothelioma cells (green, anti-cytokeratin) and 500 (5%) macrophages (red, anti-CD69) (see arrows). By fluorescent microscopy, the macrophages are found to be distributed throughout the spheroid. Spheroid is approximately 0.5 mm in diameter.

M1 M28-Macrophage Hybrid Spheroids Induce Apoptosis when Treated with TRAIL and Gemcitabine

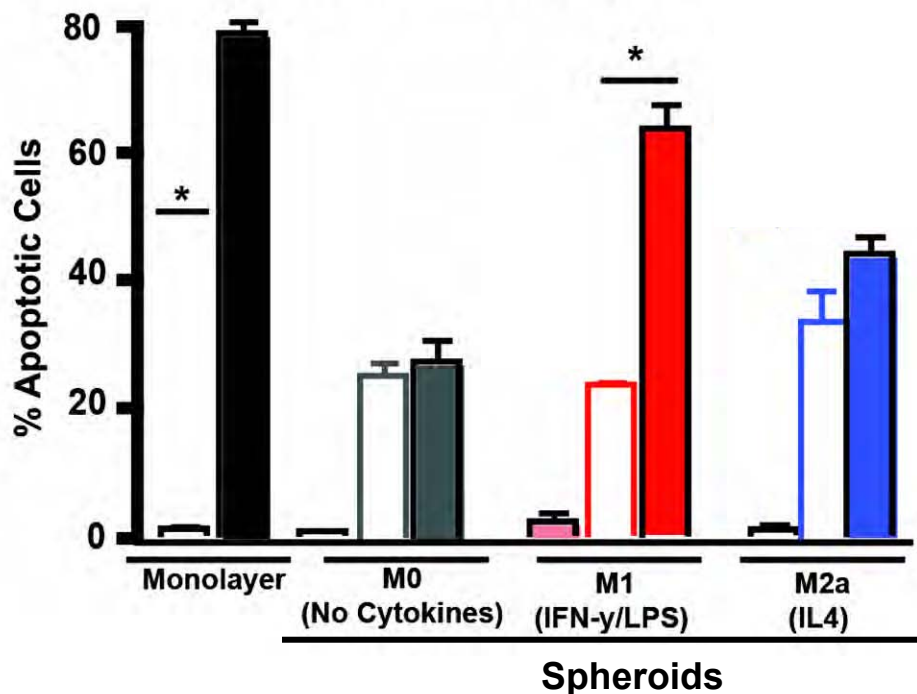


Figure 8B. Macrophages in mesothelioma spheroids can be polarized to a pro-apoptotic potential. Hybrid spheroids made with M28 mesothelioma cells and 5% THP-derived macrophages were polarized with cytokines as shown for 24 hours and then exposed to TRAIL plus gemcitabine for 24 hours. Mesothelioma cell apoptosis was increased in hybrid spheroids exposed to M1 cytokines. The degree of apoptosis was nearly equivalent to the apoptosis seen in treated monolayers (black bar), showing that the macrophage effect could significantly undermine the acquired apoptotic resistance of the 3D spheroids. (No macrophages, clear bars; with macrophages, solid gray/red/blue bars; M0, no polarizing cytokines, M1, IFN gamma/LPS; M2a, IL4). The first bars in each group are no TRAIL plus gemcitabine. The second and third bars are after TRAIL plus gemcitabine.

The Macrophage Pro-Apoptotic Effect is Cytokine-mediated

The effect of macrophages could be reproduced by use of conditioned media from macrophages polarized by different cytokine environments showing the involvement of soluble mediators. Neutralization of the cytokine tumor necrosis factor (TNF) in the conditioned media reduced the pro-apoptotic effect on the mesothelioma cells (**Figure 9**), suggesting that TNF is an important mediator of the pro-apoptotic effect of macrophages in the hybrid spheroids. A similar experiment using an antibody to IL-10 had no effect (**Figure 10**).

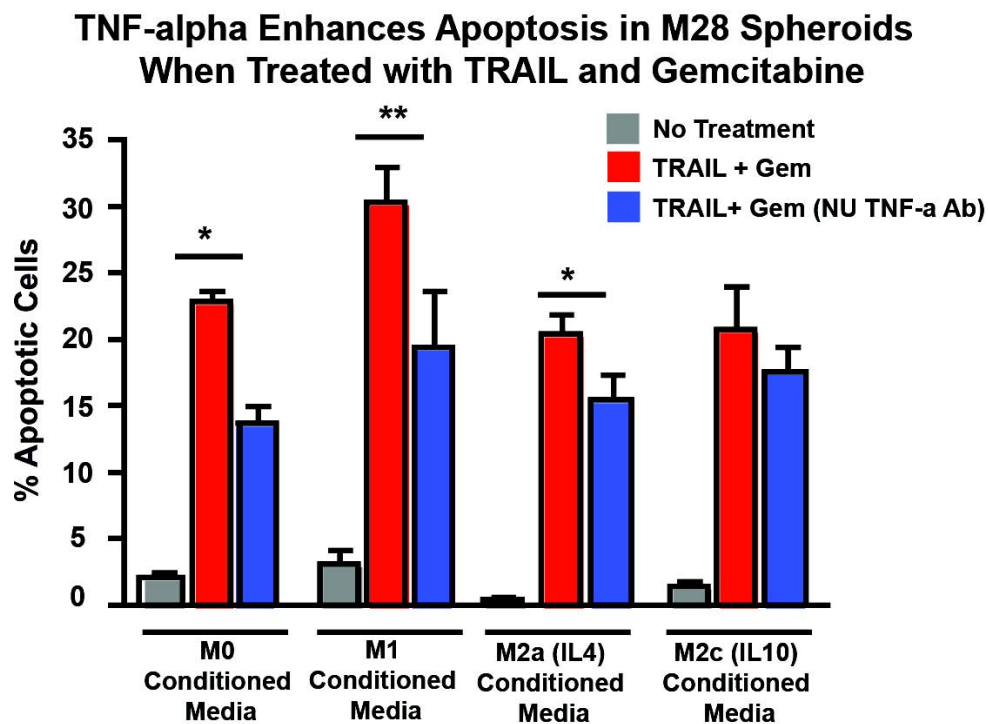
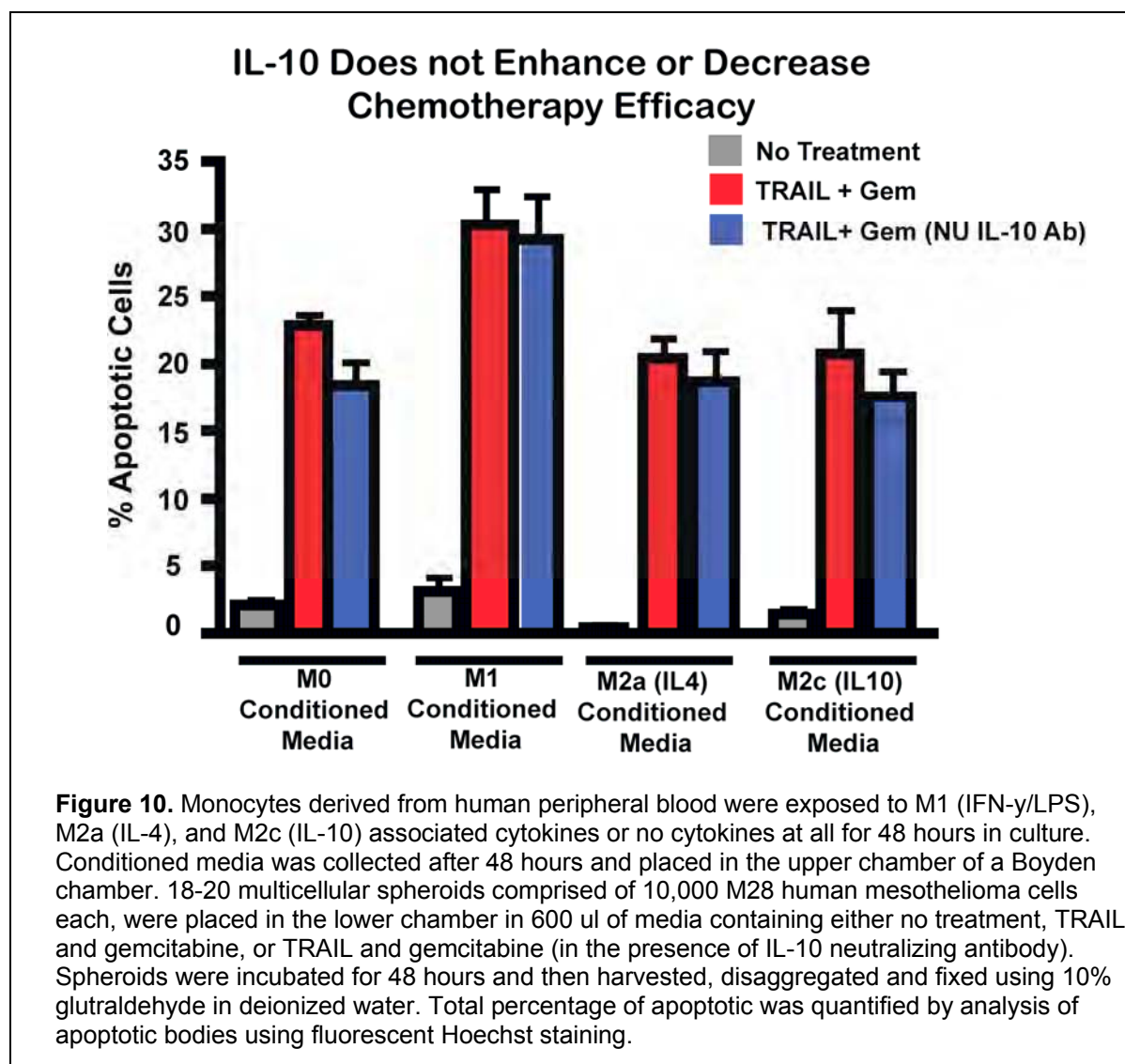


Figure 9: TNF contributes to the macrophage pro-apoptotic effect on mesothelioma cells.

Monocytes derived from human peripheral blood were exposed to M1 (IFN- γ /LPS), M2a (IL-4), and M2c (IL-10) associated cytokines or no cytokines at all for 48 hours in culture. Conditioned media was collected after 48 hours and placed in the upper chamber of a Boyden chamber. 18-20 multicellular spheroids comprised of 10,000 M28 human mesothelioma cells each, were placed in the lower chamber in 600 μ l of media containing either no treatment, TRAIL and gemcitabine, or TRAIL and gemcitabine (in the presence of TNF- α neutralizing antibody). Spheroids were incubated for 48 hours and then harvested, disaggregated and fixed using 10% glutaraldehyde in deionized water. Total percentage of apoptotic was quantified by analysis of apoptotic bodies using fluorescent Hoechst staining.



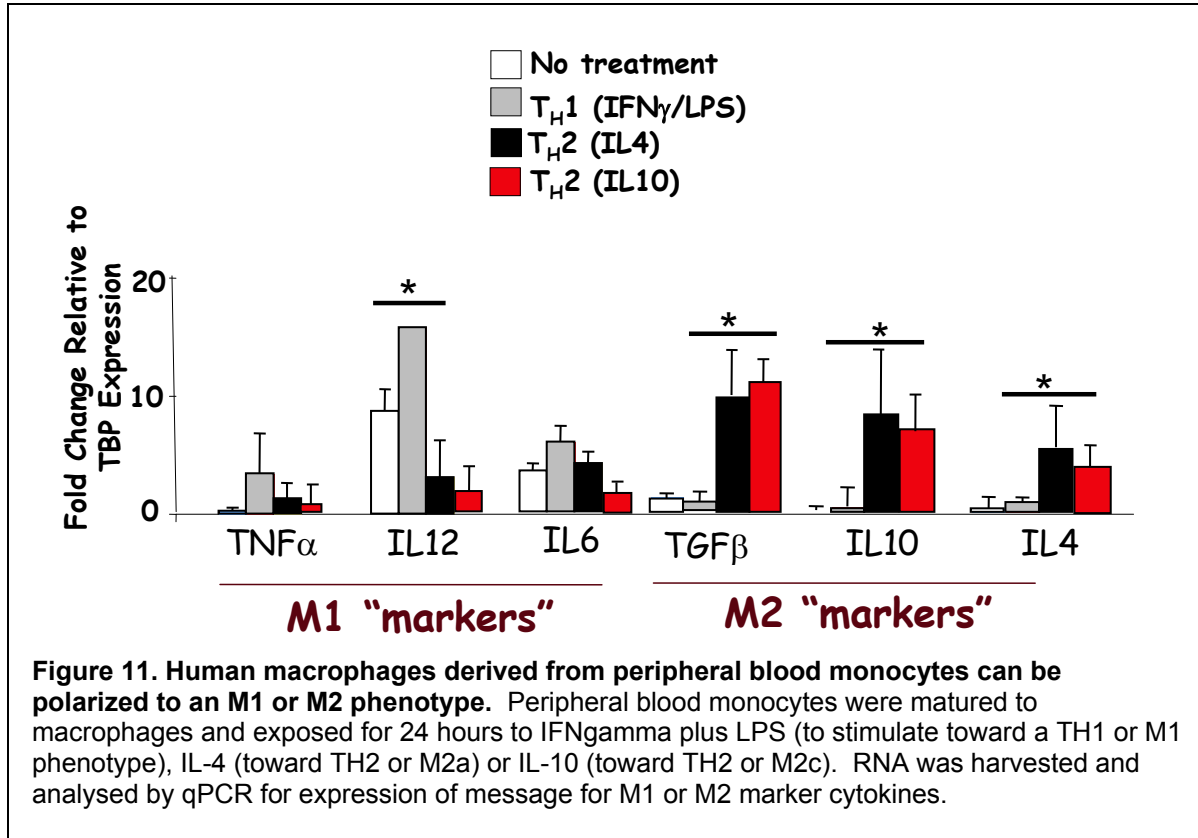
d. Analyze the effect of macrophages and of macrophage depletion on apoptosis of mesothelioma cells in tumor fragment spheroids to treatment with TRAIL or TRAIL plus gemcitabine (months 12-24).

These studies are in progress as the conditions continue to be defined in the in vitro hybrid spheroids. These conditions will then be applied to the human tumor fragment spheroids, as planned, within this next funding cycle.

e. Confirm ability of M1 cytokines to polarize macrophages to a strong M1 phenotype by exposing multicellular spheroids with macrophages (primary mesothelioma or THP-1) to M1 polarizing agents (interferon gamma plus LPS) and confirming with cytokine and gene expression assays for classics M1 or M2 polarization (months 12-24).

To confirm that we were able to reprogram macrophages, we ran qPCR on our reprogrammed peripheral blood monocytes to evaluate if key M1 and M2 markers were

expressed by the various phenotypes (**Figure 11**). M2 polarized macrophages upregulated M2 markers, such as TGF- β , IL-10, and IL-4; whereas M1 polarized macrophages upregulated markers such as TNF α and IL-12. In these and other experiments, we have not seen a significant difference in the cytokine expression of the different subsets of M2: M2a and M2c.



f. Polarize macrophages in tumor fragment spheroids using the approach found best above (months 12-24).

This task is in progress and on track to be completed during the upcoming funding cycle.

g. Determine whether repolarization of macrophages enhances apoptosis of mesothelioma cells in multicellular spheroids (grown with primary mesothelioma macrophages or THP-1 macrophages) when treated with TRAIL plus gemcitabine (months 12-24).

See **Task 2c** above showing that repolarization of THP-derived macrophages toward an M1 phenotype enhances apoptosis in MCS. In addition, experiments were performed using macrophages isolated from human mesothelioma tumor tissue (**Figure 12**). Human macrophages isolated from tumor tissue thus retain the ability to enhance apoptosis when polarized to an M1 phenotype. Such results support the feasibility of repolarizing human macrophages in vivo to an M1 and more pro-apoptotic phenotype. Human mesothelioma macrophages show the plasticity of phenotype that can potentially be harnessed to a therapeutic advantage.

M1 programmed Hybrid M28-Human Mesothelioma Macrophages Enhance Apoptosis when Treated with TRAIL and Gemcitabine

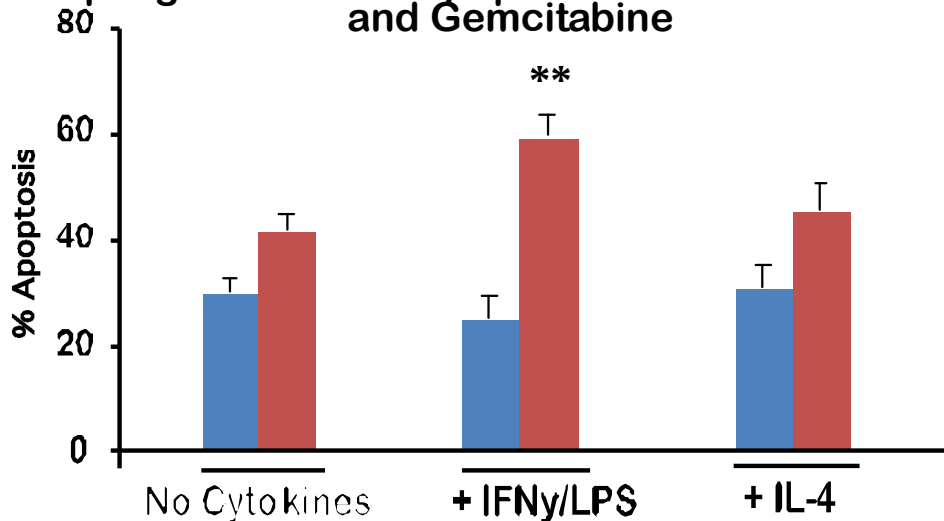


Figure 12. M1 programmed hybrid M28-Human mesothelioma macrophages enhance apoptosis when treated with TRAIL and Gemcitabine. 18-20 spheroids each comprised of 10,000 M28 human mesothelioma cells alone (*blue bars*) were treated with polarizing cytokines of either (a) M0 – no cytokines (b) M1 – IFN- γ /LPS or (c) M2a – IL-4. In parallel to these spheroids, hybrid spheroids (*red bars*) comprised of 9,500 M28 mesothelioma cells and 500 macrophages isolated and differentiated from human malignant pleural mesothelioma tumors were treated with polarizing cytokines of either (a) M0 – no cytokines (b) M1 – IFN- γ /LPS or (c) M2a – IL-4 for 24 hours. These hybrid spheroids were then treated with TRAIL and gemcitabine for 24 hours in addition to their respective polarizing cytokines. Hybrid and M28 spheroids were then harvested, fixed and analyzed for apoptosis using Hoescht staining to quantify the total % of apoptotic cells. (*Blue, no macrophages; red, with macrophages*) (**, $p < 0.05$ significant increase in apoptosis in hybrid spheroids polarized to M1 compared to M0 or M2 phenotype)

h. Determine whether repolarization of macrophages enhances apoptosis of mesothelioma cells in tumor fragment spheroids (months 12-24).

In progress.

Task 3. Define functional significance of macrophage depletion or repolarization on mesothelioma survival in vivo.

a. Characterize immune cell profile of the murine mesothelioma model induced by intraperitoneal asbestos injections in the NF2+/- mouse model (months 0-24).

We have begun the mesothelioma model in which NF2+/- mice, with a genetic predisposition for the development of asbestos-induced mesothelioma, are injected every 3 weeks with crocidolite asbestos and followed many months for the development of mesothelioma, as reported. We currently have a cohort of 20 mice that have completed the 8 injections and we have an additional cohort of 30 mice that are in the process of receiving the 8 asbestos injections.

b. Deplete in vivo macrophages with intraperitoneal clodronate-liposomes in mice without mesothelioma to establish protocol (months 4-12).

We have followed the in vivo protocol for the use of intraperitoneal clodronate for macrophage depletion. In GFP-macrophage mice, the clodronate was able to eliminate the GFP signal and all cells costaining with two macrophage markers, CD11b+ and F4/80. Thus, the clodronate is effective in removing macrophages when introduced on the schedule as reported. In the first in vivo mesothelioma study (see Task 3d below), clodronate over 3 injections (day 0, 5, 10), led to a depletion of macrophages in the peritoneal lavage by flow cytometric analysis of macrophage markers. Further studies in mice with mesothelioma and in vitro tumor fragment spheroids and multicellular spheroids will be needed to confirm that this regimen of clodronate also removes macrophages from tumor tissues.

c. Deplete in vivo macrophages in mice with mesothelioma to confirm depletion, to establish effect on other immune cell populations, and to confirm lack of toxicity on the mice (months 12-24).

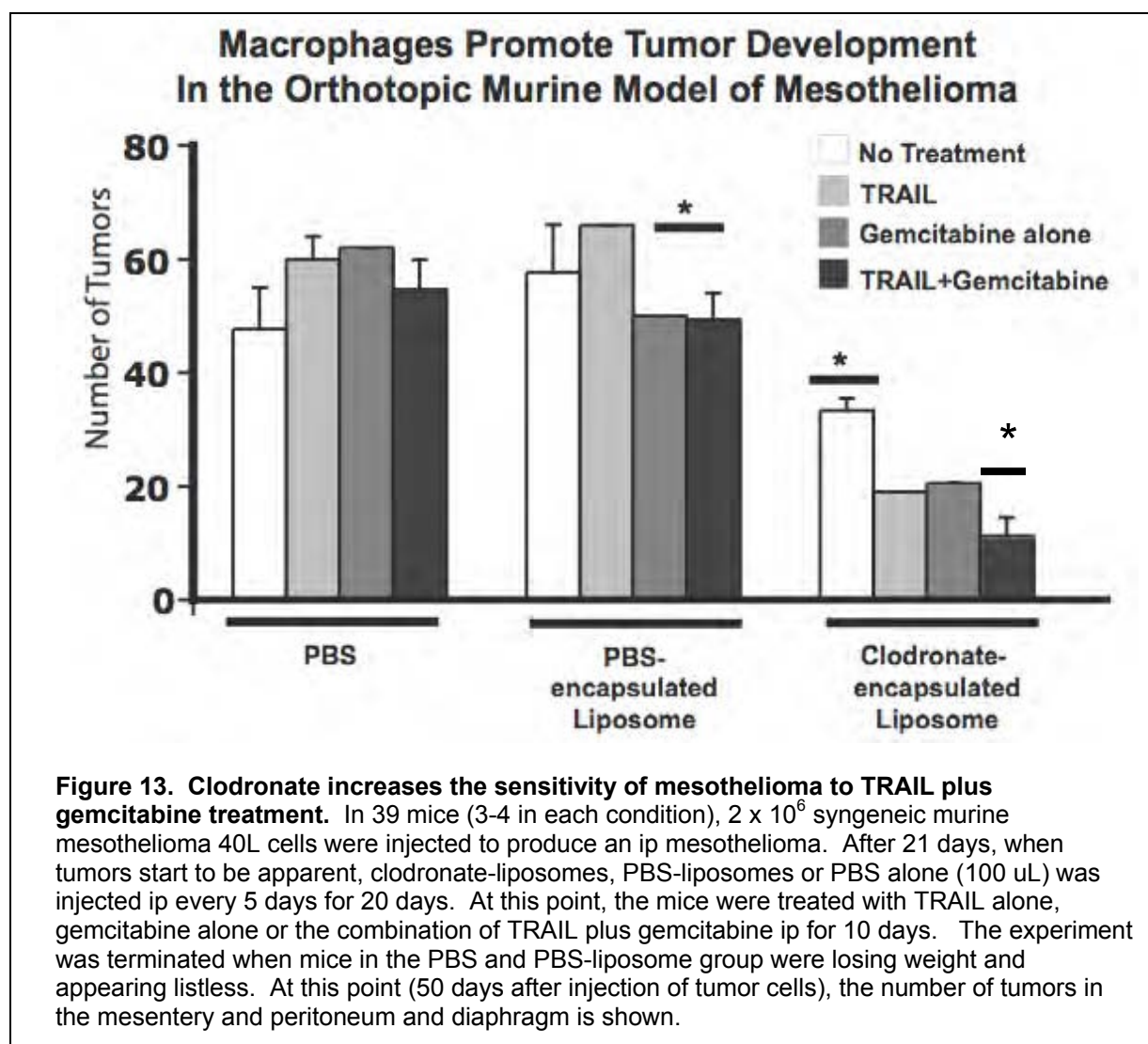
In the 40L mesothelioma mice, the tumor cells were injected at day 0 and clodronate was started at day 21 and continued every 5 days until day 40. A FACS analysis at day 40 before treatment began with TRAIL plus gemcitabine showed a significant reduction in macrophages. From this in vivo experiment (see Task 3d below), we have ascites fluid stored and fixed in preparation for flow studies and tissue fixed for immunohistochemistry that will provide information on the effect of clodronate and of TRAIL plus gemcitabine on immune cell populations. In our first experiments with clodronate, there was no evidence of toxicity. In fact, the clodronate-treated mice ultimately did better than the other mice with fewer tumors and appeared healthier. Our next in vivo studies will utilize blood creatinine measurements, weekly weights and other metrics to monitor for any toxicity of the clodronate.

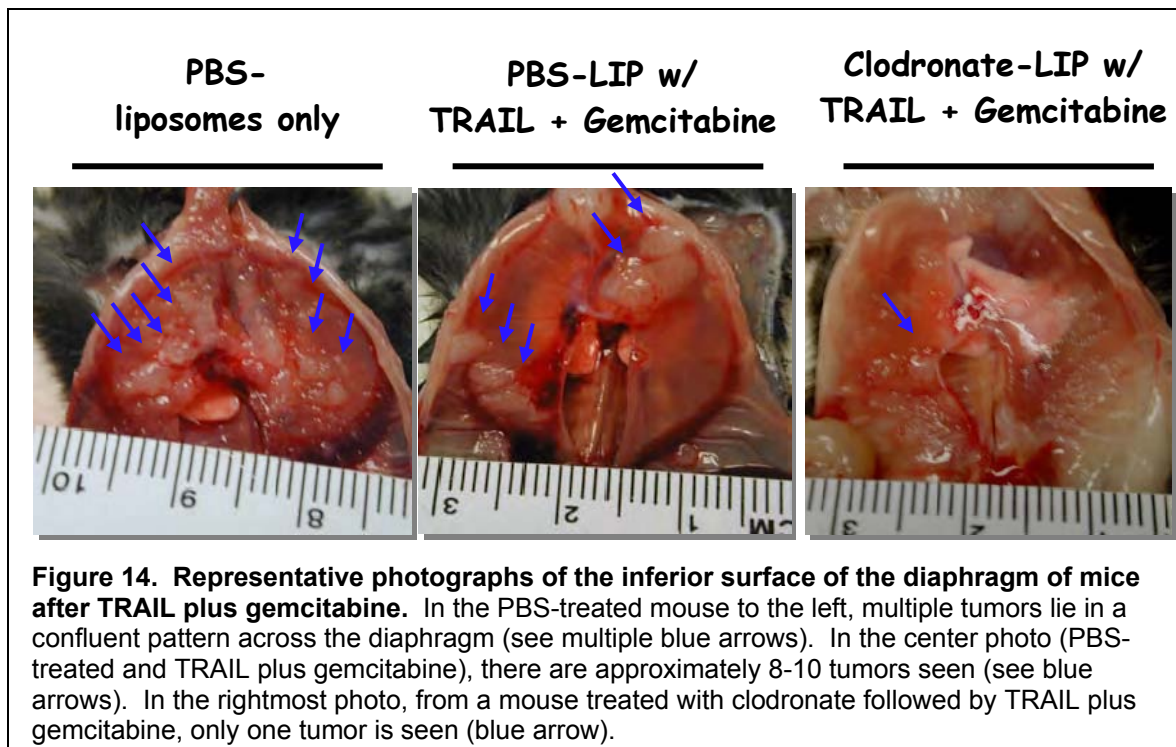
d. Determine whether macrophage depletion in mice with mesothelioma enhances apoptotic and therapeutic response of mice with mesothelioma to TRAIL plus gemcitabine over 1-4 weeks after treatment (months 24-36).

Because the NF2 +/- asbestos mesothelioma model takes many months to develop, we have begun a second model of mesothelioma in immunocompetent mice produced by the IP injection of a syngeneic murine mesothelioma line (40L) in C57/Bl/6 mice. In this model, intraperitoneal mesothelioma forms 3-4 weeks after the ip injection of 2×10^6 40L

cells. This model was developed by Dr. Agnes Kane, who kindly provided this cell line and a second line (7) for these studies [9]. These more rapid and reproducible models will enable us to study normal inflammatory cell recruitment and manipulation and to refine our treatment dosing and imaging in preparation for the more challenging, expensive and time-consuming but ultimately more relevant asbestos-induced NF+/- mouse model. The first studies in the 40L peritoneal mesothelioma model have already yielded exciting results.

We have performed one experiment in mice with the 40L peritoneal mesothelioma, using clodronate-liposomes and then TRAIL plus gemcitabine to learn whether the clodronate-mediated depletion of macrophages will sensitize the tumor cells to chemotherapy. We have found that the mice treated with clodronate had smaller tumors at 20 days, as had been reported by Dr. Kane's group [9]. However, we have extended these studies to show that the depletion of the macrophages sensitizes the tumor to chemotherapy, leading to mice with smaller, fewer tumors or in some cases no visible tumor at all (Figure 13 and 14).





These studies have led to several plans for further experiments. The 40L cells are now being transfected with a luciferase construct so that, in the next experiments, the tumor size can be monitored by luciferase optical imaging. This will also be done with the second cell line, the 7 cells, so that additional experiments with a second cell line can be used to confirm these findings.

Cells, spheroids and tissues have been collected to be analyzed by FACS for immune cell populations, RNA for cytokine expression, immunohistochemistry for macrophage numbers and for apoptosis (cleaved caspase 3) as well as for confirmation of tumor morphology and burden.

e. Repolarize macrophages in vivo by injections of interferon gamma plus LPS and assaying cytokines of peritoneal ascites and gene expression of peritoneal macrophages obtained by lavage to confirm method of repolarization, and to select best tolerated method for the mice (months 12-24).

We are on target to accomplish this task by the third funding cycle.

f. Determine whether repolarization of macrophages in vivo enhances the apoptotic and therapeutic response of mice with mesothelioma to TRAIL plus gemcitabine over 1-4 weeks after treatment (months 24-36).

We are on target to accomplish this task in the third funding cycle.

g. Determine whether the depletion or repolarization of macrophages enhances survival of mice with mesothelioma after treatment with vehicle, TRAIL or TRAIL plus gemcitabine (months 24-36).

We are on target to accomplish this task in the third funding cycle.

III. KEY RESEARCH ACCOMPLISHMENTS

Task 1. To determine the functional significance of macrophage phenotype on mesothelioma cell survival.

- a. Elucidate the percentage of immune cells (CD45+) in human mesothelioma tumors and correlate immune cell infiltration with histopathologic subtype (months 1-6).
 - Acquired 71 fixed and paraffin-embedded mesothelioma tumor samples
 - Prepared mesothelioma tumor tissue microarrays for immunohistochemistry
 - Determined the pathologic subtype of each tumor in concert with the thoracic pathologist, Dr. Steven Nishimura
 - Quantified each sub-population as a percentage of the total inflammatory cell population using digital imaging software, the Aperio system, optimized for either membrane or nuclear staining
 - Correlated presence of inflammatory cell populations with mesothelioma pathologic subtype
 - Established collaboration with a pulmonary laboratory of Dr. Michael Matthay to obtain normal human pleura
- b. Determine the macrophage population (CD14+) as a percentage of the total immune cell population by flow cytometry (months 1-36).
 - Optimized disaggregation protocol for mesothelioma tumors
 - Analysed the CD14+ macrophage population in 26 fresh tumors
 - Established that the percentage of CD14+ cells in the mesothelioma inflammatory population exceeds that of the other thoracic tumors, lung and esophagus
 - Expanded flow cytometry studies to a new high density 14 color system
 - Confirmed the high percentage of macrophages using high density flow
 - Analyzed 26 fresh tumors using 5 color flow cytometry
 - Analyzed 5 tumors so far using 14 color, high density flow cytometry
 - Initiated collection of peripheral blood samples from the patients with mesothelioma to compare activation status of cells in tumor compared to periphery
 - Included mesothelin in panel of cell surface markers to quantify percentages of mesothelioma cells in tumors
- c. Determine the profile of other immune cells within the microenvironment of the mesothelioma tumor using a panel of cell surface markers (months 1-36).
 - Performed immunohistochemical analysis on 71 tumors to identify inflammatory cell infiltrates, e.g. CD45, CD68, CD8, CD4 and CD20
- d. Confirm macrophage percentages by immunodetection of the same mesothelioma in fixed tissues (CD68+) (months 1-36).
 - Confirmed the percentages of macrophages in mesothelioma using same tumors studied in immunohistochemistry (by CD68+) and in flow cytometry (by CD14+)
- e. Isolate macrophages from human mesothelioma disaggregated into single cells by flow cytometry for use in co-culture spheroids with mesothelioma cells (1-12).
 - Isolated macrophages from fresh mesothelioma tumors with high viability (80-90%) and in sufficient numbers to study
 - Produced hybrid spheroids with macrophages isolated from human mesothelioma tumors

f. Determine macrophage functional properties in mesothelioma using fixed tissues, by tissue microarray, and by immunohistochemistry for protein expression to define their M1 or M2 microenvironmental status (months 1-12)

- Stored mesothelioma tissues in tissue bank for these studies while technique is being tested in laboratory

g. Determine the M1 or M2 gene expression signature of macrophages by commercial global chip assay for RNA from tumor tissue or cultures of cells/spheroids for gene profile (months 1-12).

- Quantified cytokine message in 7 snap frozen mesothelioma tumors
- Compared message to 4 normal pleural samples

h. Analyze cytokines produced by mesothelioma tumor fragments by commercial cytokine bead assay from human mesothelioma grown as tumor fragments (months 1-12).

- Determined that cytokines were secreted by tumor fragment spheroids, but were too dilute for accuracy
- Initiated testing of an intracellular cytokine assay
- Developed mesothelin staining and gating by flow cytometry for use in this intracellular and other flow cytometry studies to identify mesothelioma cell population

i. Analyze cytokines produced by multicellular spheroids made from either mesothelioma cells alone or mesothelioma cells plus macrophages (THP-differentiated) (months 1-12).

- Quantified expression of key M1 or M2 cytokines in multicellular spheroids e.g M1 (TNF, IL-12, IL-6) or M2 (IL-4, IL-10, TGF) compared to housekeeping gene TBP
- Determined that the polarization protocol does polarize hybrid spheroids to an M1 or M2 phenotype

j. Analyze changes in cytokine expression when spheroids (mesothelioma only or mesothelioma plus macrophage) are treated with TRAIL or TRAIL plus gemcitabine (months 1-12).

- Determined that treatment with TRAIL or TRAIL plus gemcitabine has no significant effect on the M1 or M2 phenotype

Task 2. To determine the functional significance of macrophages as regulators of mesothelioma apoptosis in vitro.

a. Confirm ability of clodronate-liposomes to deplete macrophages from multicellular spheroids made either with THP-1 differentiated macrophage cells or with primary mesothelioma-derived macrophages and determine optimal dose and timing (months 9-14).

- Obtained and prepared clodronate-liposomes for in vivo use
- Use in vitro systems not initiated in months 1-12

b. Confirm ability of clodronate-liposomes to deplete macrophages from mesothelioma tumor fragment spheroids with dose and timing established above (months 12-24).

- Obtained and prepared clodronate-liposomes for in vivo use
- Use in vitro systems not initiated in months 1-12

- c. Analyze effect of macrophages and of macrophage depletion on apoptosis of mesothelioma cells to treatment with TRAIL or TRAIL plus gemcitabine using multicellular spheroids either with no macrophages, macrophage-depleted or with macrophages (months 12-18).
- Derived macrophages from peripheral blood and THP monocyte-like cells
 - Produced hybrid spheroids with two different mesothelioma cell lines (REN, M28) combined with macrophages derived from 4 different sources (THP, peripheral blood, banked blood, mesothelioma tumors)
 - Established an imaging protocol to show that macrophages were viable and well distributed within spheroids
 - Determined that macrophages alone (without polarization) had no consistent effect on the mesothelioma cell apoptotic response to treatment
- d. Analyze the effect of macrophages and of macrophage depletion on apoptosis of mesothelioma cells in tumor fragment spheroids to treatment with TRAIL or TRAIL plus gemcitabine.
- Not initiated in months 1-12. Plan to use optimal protocol worked out in Task 2c in multicellular, hybrid spheroids
- e. Confirm ability of M1 cytokines to polarize macrophages to a strong M1 phenotype by exposing multicellular spheroids with macrophages (primary mesothelioma or THP-1) to M1 polarizing agents (interferon gamma plus LPS) and confirming with cytokine and gene expression assays for classics M1 or M2 polarization (months 12-24).
- Determined that M1 stimulation (IFN gamma plus LPS) does polarize macrophages to an M1 phenotype (e.g. expressing TNF, IL12 and IL6)
 - Determined that M2 stimulation (IL4 - M2a or IL10 - M2c) does polarize macrophages to an M2 phenotype (e.g. expressing TGF beta, IL10 and IL4)
- f. Polarize macrophages in tumor fragment spheroids using the approach found best above (months 12-24).
- Not initiated in months 1-12.
- g. Determine whether repolarization of macrophages enhances apoptosis of mesothelioma cells in multicellular spheroids (grown with primary mesothelioma macrophages or THP-1 macrophages) when treated with TRAIL plus gemcitabine (months 12-24).
- Determined that the presence of M1 polarized macrophages consistently enhances mesothelioma cell apoptotic responses to TRAIL plus gemcitabine
 - Showed that this conclusion does not depend on the source of macrophages although peripheral blood monocyte and THP-derived macrophages appear most robust
 - Showed, in expanded studies, that this pro-apoptotic effect was mediated by soluble factors and could be reproduced by exposing the mesothelioma cells to media conditioned by M1 polarized macrophages
 - Determined that the cytokine TNF (an M1 cytokine), but not IL-10 (an M2 cytokine), contributed to the macrophage pro-apoptotic effect
- h. Determine whether repolarization of macrophages enhances apoptosis of mesothelioma cells in tumor fragment spheroids (months 12-24).
- Not initiated in months 1-12

Task 3. Define functional significance of macrophage depletion or repolarization on mesothelioma survival in vivo.

- a. Characterize immune cell profile of the murine mesothelioma model induced by intraperitoneal asbestos injections in the NF2+/- mouse model (months 0-24).
 - Established an NF2+/- mouse cohort of 20 mice that have completed the required 8 every 3 weeks intraperitoneal asbestos injections for production of mesothelioma
 - Initiated a further cohort of 30 mice that are in process of receiving the 8 asbestos ip injections
 - Established a second immunocompetent mouse model of mesothelioma using the 40L murine mesothelioma cell line obtained from Dr. Agnes Kane
 - Carried out one experiment in the 40L mesothelioma model showing that mesothelioma develops in 4 weeks, as reported by Dr. Kane
 - Characterization of immune cell population in these two models (asbestos induced NF2+/- and syngeneic orthotopic 40L) are planned for months 12-36
- b. Deplete in vivo macrophages with intraperitoneal clodronate-liposomes in mice without mesothelioma to establish protocol (months 4-12).
 - Depleted intraperitoneal macrophages successfully using clodronate-embedded liposomes from GFP-macrophage labeled mice by showing loss of GFP signal and loss of cells co-staining with F4/80 and CD11b+
- c. Deplete in vivo macrophages in mice with mesothelioma to confirm depletion, to establish effect on other immune cell populations, and to confirm lack of toxicity on the mice (months 12-24).
 - Used clodronate-liposomes in mice with 40L orthotopic mesothelioma
 - Collected tissues after clodronate treatment to determine whether clodronate used in this schedule depleted all tumor-associated macrophages
- d. Determine whether macrophage depletion in mice with mesothelioma enhances apoptotic and therapeutic response of mice with mesothelioma to TRAIL plus gemcitabine over 1-4 weeks after treatment (months 24-36).
 - Carried out one experiment in which mice implanted ip with 40L syngeneic mesothelioma cells were first given clodronate-liposomes (or PBS or PBS-liposomes) intraperitoneally and then treated with nothing, TRAIL alone, gemcitabine alone or TRAIL plus gemcitabine
 - Showed that clodronate-treatment itself was associated with a lower tumor burden
 - Found that clodronate-treatment increased the efficacy of the treatment with TRAIL plus gemcitabine
 - Collected ascites and tissues for determining presence of macrophages, other immune cells, tumor burden and apoptotic cells
- e. Repolarize macrophages in vivo by injections of interferon gamma plus LPS and assaying cytokines of peritoneal ascites and gene expression of peritoneal macrophages obtained by lavage to confirm method of repolarization, and to select best tolerated method for the mice (months 12-24).
 - Not initiated in months 1-12

f. Determine whether repolarization of macrophages in vivo enhances the apoptotic and therapeutic response of mice with mesothelioma to TRAIL plus gemcitabine over 1-4 weeks after treatment (months 24-36).

- Not initiated in months 1-12

g. Determine whether the depletion or repolarization of macrophages enhances survival of mice with mesothelioma after treatment with vehicle, TRAIL or TRAIL plus gemcitabine (months 24-36).

- Not initiated in months 1-12

IV. REPORTABLE OUTCOMES

A. MANUSCRIPTS

Months 1-12 (Provided as Appendix Material in this Progress Report)

- DeNardo DG, Andreu P, **Coussens LM**. (2010) Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity. *Cancer Metastasis Rev*, 29(2):309-316, [PMID: 20405169](#)

B. ABSTRACTS

Months 1-12 (Provided as Appendix Material in this Progress Report)

C. PRESENTATIONS

Symposia and Workshops: International

Symposia and Workshops: National

- | | |
|------|--|
| 2009 | Broaddus, V. Courtney. <i>Role of macrophages in apoptotic resistance of mesothelioma</i> . In Workshop on Preclinical Drug and Target Discovery Pipeline. International Mesothelioma Program, Brigham and Womens Hospital, Harvard Medical School, Boston, MA, USA. |
| 2010 | Coussens, Lisa M. PLENARY LECTURE . <i>Regulation of protumor immunity and cancer development</i> . 2010 Annual Meeting of the American Association for Cancer Research, Washington DC USA. |
| 2010 | Jablons, David M. <i>Exploiting emerging biology for the treatment of malignant mesothelioma</i> . In Symposium entitled: Translational Initiatives in Mesothelioma. American Association of Cancer Research, Washington DC, USA. |
| 2010 | Coussens, Lisa M. <i>Regulation of protumor immunity and cancer development</i> . IN: <i>3rd Annual Wyeth Discovery Frontiers in Human Disease Symposium</i> , New York, NY USA |

Invited Lectures/Seminars: International

Invited Lectures/Seminars: National

- 2010 Coussens, Lisa M. *Inflammation and cancer: polarized immune responses regulate cancer development*. Cold Spring Harbor Laboratory, CSH NY USA
- 2010 Broaddus, V. Courtney. *Current studies under the DOD grant mechanism: macrophages and their contribution to mesothelioma*. Mesothelioma Applied Research Foundation, Washington, DC, USA

Presentations by Coussens or Broaddus Lab Members:

Nikita Kolhatkar (Coussens and Broaddus' lab, pre-doctoral student)

- 2009 Kohatkar, Nikita. *Macrophages contribute to mesothelioma chemoresistance*. Poster presentation. Annual UCSF Pulmonary Research Retreat, San Francisco, CA.
- 2010 Kohatkar, Nikita. Targeting macrophages as a novel therapeutic approach for malignant pleural mesothelioma. Invited presentation. American Association for Cancer Research. Washington, DC.

D. PATENTS AND LICENSES: None

E. DEGREES OBTAINED: None

F. REAGENT DEVELOPMENT:

- Preparation of tumor tissue microarrays prepared with 71 mesothelioma tumors
- Preparation of tumor tissue microarrays stained for CD68, CD4, CD8 and a multitude of inflammatory markers
- Generation of hybrid multicellular spheroids with macrophages derived from THP cell lines
- Generation of hybrid multicellular spheroids with macrophages derived from peripheral blood monocytes
- Generation of hybrid multicellular spheroids with macrophages derived from macrophages isolated from fresh human mesothelioma
- Collection of peripheral blood from patients with mesothelioma
- Collection of frozen mesothelioma tissue from patients at the time of surgery to be used for RNA extraction and qPCR

G. FUNDING APPLIED FOR BASED ON WORK SUPPORTED BY THIS FUNDING:

- Barbone, Dario. Mesothelioma Applied Research Foundation. Pending
- Barbone, Dario. SPORE mechanism Career Development Award. Pending.

H. EMPLOYMENT/RESEARCH OPPORTUNITIES APPLIED FOR:

- Kolhatkar, Nikita. Accepted to a postdoctoral research program in immunology at The University of Washington, Seattle, WA.

V. CONCLUSION

Inflammation is now recognized as promoting solid tumor growth and survival. The inflammatory cells release cytokines and soluble mediators that *directly* promote growth and survival of the malignant cells, and *indirectly* support the tumor by inducing angiogenesis or by suppressing effective cytotoxic T-cell functions. Each tumor now appears to have a unique inflammatory cell milieu. From our studies to date, mesothelioma appears to have an innate inflammatory profile, with a predominance of macrophages and neutrophils, whereas the other thoracic malignancies studied have a more adaptive profile.

The macrophage is the major inflammatory cell type in mesothelioma and, as such, may play a powerful role in mesothelioma development, maintenance and resistance to chemotherapy. Our studies to date have not yet shown the phenotype of these tumor-associated macrophages within their complex microenvironment although we have been able to isolate the macrophages to demonstrate that they retain plasticity and can be polarized to an M1 or an M2 phenotype. We have also completed studies showing that macrophages, when polarized to an M1 phenotype, alter the sensitivity of the mesothelioma cells to therapy. This sensitization to chemotherapy does not require contact but can be reproduced by conditioned media from the macrophages.

Our most exciting preliminary data comes from the first study in immunocompetent mice with orthotopic syngeneic mesothelioma cells. Using clodronate to deplete macrophages, we found that the tumors that formed were smaller (as reported by Dr. Kane's group) but were also more sensitive to subsequent chemotherapy. This preliminary study shows the exciting potential for manipulation of the macrophage in the tumor environment. If depletion of the macrophages can produce a significant improvement in mesothelioma treatment in this one model, then polarization to an M1 phenotype may have even a greater benefit.

In our second funding cycle, we will aim to answer more of the intriguing questions about the role of the macrophage in mesothelioma and about our ability to block it or manipulate it to improve chemoresponsiveness. We will focus on the in vitro hybrid and ex vivo tumor fragment models to identify the molecular mechanisms of the macrophage-mesothelioma interaction and on our two in vivo models of mesothelioma (40L syngeneic orthotopic and NF2+/- -asbestos-induced) to alter macrophage function. The macrophage, the predominant inflammatory cell in all the subtypes of mesothelioma, is already infiltrated throughout the tumor mass. Manipulation of this powerful cell type has the potential to transform mesothelioma from a chemoresistant, intractable and incurable tumor to one more sensitive to current or novel therapies.

VI. BIBLIOGRAPHY

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2. Hill, R.J., R.E. Edwards, and P. Carthew, *Early changes in the pleural mesothelium following intrapleural inoculation of the mineral fibre erionite and the subsequent development of mesotheliomas*. J. Exp. Pathol., 1990. **71**(1): p. 105-118.

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3. Fennell, D.A. and R.M. Rudd, *Defective core-apoptosis signalling in diffuse malignant pleural mesothelioma: opportunities for effective drug development*. Lancet Oncol., 2004. 5(6): p. 354-362.
4. Pollard, J.W., *Macrophages define the invasive microenvironment in breast cancer*. J. Leukocyte Biol., 2008. 84: p. 1-8.
5. Dave, S.S., et al., *Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells*. N. Engl. J. Med., 2004. 351: p. 2159-2169.
6. Tsutsui, S., et al., *Macrophage infiltration and its prognostic implications in breast cancer*. Oncol. Rep., 2005. 14(2): p. 425-431.
7. Johansson, M., D.G. Denardo, and L.M. Coussens, *Polarized immune responses differentially regulate cancer development*. Immunol. Rev., 2008. 222: p. 145-154.
8. Montovani, A., A. Sica, and M. Locati, *New vistas on macrophage differentiation and activation*. Eur. J. Immunol., 2007. 37: p. 14-16.
9. Miselis, N.R., et al., *Targeting tumor-associated macrophages in an orthotopic murine model of diffuse malignant mesothelioma*. Mol. Cancer Ther., 2008. 7(4): p. 788-799.

VII. APPENDICES

- A. Complete academic curriculum vitae for Dr. V. Courtney Broaddus and Dr. Lisa Coussens
- B. Publications for months 1-12
- C. Abstracts for months 1-12

July 2010

**UNIVERSITY OF CALIFORNIA SAN FRANCISCO
CURRICULUM VITAE**

Name: V. Courtney Broaddus

Position: Professor of Medicine, Step 3
Department of Medicine
School of Medicine

Address: Lung Biology Center
San Francisco General Hospital
UCSF Box 0841
San Francisco, CA 94110-0841
Voice: (415) 206-3513
Fax: (415) 206-4123
Email: cbroaddus@medsfgh.ucsf.edu
<http://pulmonary.ucsf.edu/faculty/broaddus.html>

EDUCATION:

1971-1975	Duke University, Durham, NC	B.S.	Summa Cum Laude, Zoology
1975-1979	University of Pennsylvania	M.D.	Medicine
1979-1980	University of Pennsylvania	Intern	Medicine
1980-1982	University of Pennsylvania	Resident	Medicine
1983-1986	University of California, San Francisco	Fellow	Pulmonary/ Critical Care Medicine
2001-2002	Comprehensive Cancer Center, UCSF	Sabbatical	
			Laboratory of Gerard Evan, Ph.D.
2007	Leadership Development for Physicians in Academic Health Centers		
			Harvard School of Public Health

LICENSING AND CERTIFICATION

1982	California License	G-049379
1982	Internal Medicine	American Board of Internal Medicine
1986	Pulmonary Disease	American Board of Internal Medicine
1999-2009	Critical Care Medicine	American Board of Internal Medicine

PRINCIPAL POSITIONS HELD

1986-1988	University of California, SF	Instructor in Residence	Medicine
1988-1995	University of California, SF	Assistant Professor in Residence	Medicine
1995-1997	University of California, SF	Associate Professor in Residence	Medicine
1997-2001	University of California, SF	Associate Professor	Medicine
2001-now	University of California, SF	Professor	Medicine

OTHER POSITIONS HELD CONCURRENTLY

1998-now	Dept of Medicine	Chief, Division of Pulmonary and Critical Care Medicine, SFGH
2004-now	Dept of Medicine	Associate Director, Lung Biology Center, SFGH

HONORS AND AWARDS

1974	Phi Beta Kappa, Junior Year
1975	Summa Cum Laude
1978	Alpha Omega Alpha, Junior Year
1979	Janet M. Glasgow Memorial Award and Citation
1985	Individual National Research Service Award, NIH
1987	Clinical Investigator Award, NHLBI
1988	American Physiological Society, Elected
1991	Distinction in Teaching Award, Academic Senate, UCSF
1991	Associate, Scientific Staff, Cardiovascular Research Institute, Elected
1992	Pre-tenure Award, UCSF
1999-2002	President, International Mesothelioma Interest Group
2003	Nomination for Most Outstanding Teacher, UCSF Women In Medicine
2005-now	Best Doctors in America, Selected
2005	Faculty of the Year Award, SFGH Association of Business Officers' Group
2006	Western Society of Clinical Investigation, Elected
2007&2008	Nomination for Subspecialist Consultant of the Year Award, SFGH
2008	Nomination for Wagner Award, International Mesothelioma Interest Group
2010	Michael S. Stulbarg Outstanding Teaching Award, UCSF Pulmonary
2010	Pioneer Award, The Mesothelioma Applied Research Foundation.

KEYWORDS/AREAS OF INTEREST

Apoptosis, mesothelioma, macrophages, synergy, 3-dimensional models, TRAIL (TNF-related apoptosis-inducing ligand), pleural disease, pleural effusions, asbestos.

PROFESSIONAL ACTIVITIES

CLINICAL

Attending, Medical Intensive Care Unit, SFGH: I attend 1 month of the year on the ICU, 7 days a week, supervising 8 senior residents and interns.

Attending, Pulmonary Consult Service, SFGH: I attend 1 month each year seeing inpatients with pulmonary problems, 5 days a week, supervising 2 fellows and 1-2 residents/medical students.

PROFESSIONAL ORGANIZATIONS

Memberships

Lung Biology Center, San Francisco General Hospital
 Thoracic Oncology Research Group, UCSF Cancer Center
 Cardiovascular Research Institute, University of California, San Francisco
 Western Society of Clinical Investigation
 American Thoracic Society
 California Thoracic Society
 International Mesothelioma Interest Group
 Mesothelioma Applied Research Foundation
 International Association for the Study of Lung Cancer (IASLC)

Service to Professional Organizations

1988-1992	ALA/American Thoracic Society	Research Fellowship Review Committee
1989-1991	ALA/American Thoracic Society	Manpower Review Committee
1991-1992	American Thoracic Society	Program Committee, Respiratory Structure and Function
1993-1994	American Thoracic Society	Nominating Committee, Respiratory Cell and Molecular Biology
1994-1995	American Thoracic Society	Women's Affairs Committee
1994-1997	American Thoracic Society	Program Committee, Respiratory Cell and Molecular Biology
1995-1997	California Lung Association	Research Fellowship Committee
1995-2001	American Thoracic Society	Long Range Planning Committee, Respiratory Cell and Molecular Biology
1995-1997	American Thoracic Society	Program and Budget Committee
1995-1999	Am Federation for Med Research	UCSF Representative
1997-2003	American Lung Association of CA	Research Administrative Committee
1999-2002	Intl Mesothelioma Interest Group	President
1999-2001	American Thoracic Society	Chair, Long Range Planning Committee, Respiratory Cell and Molecular Biology
2000-2002	American Thoracic Society	Planning on ATS Research Agenda
2000-2002	American Thoracic Society	Assembly Structure Task Force
2003-2004	American Thoracic Society	Education Committee
2003-2007	American Thoracic Society	Scientific Advisory Committee
2005-now	International Mesothelioma Interest Group	Member, Board of Directors Scientific Organizing Committee
2006-now	Intl Mesothelioma Interest Group	Chair, Website Subcommittee
2007-2009	American Thoracic Society	Chair, Nominating Committee, Respiratory Cell and Molecular Biology
2007-2008	American Thoracic Society	Member, Search Committee for Editor, Am. Journal of Respiratory Cell Mol. Biol.
2008-now	Mesothelioma Applied Research Foundation	Member, Scientific Advisory Board

SERVICE TO PROFESSIONAL PUBLICATIONS

1990-now	Ad hoc referee for: Oncogene (2 papers in last year) American Journal of Pathology (1 paper in last year) New England Journal of Medicine (2 papers in last year) American Journal of Respiratory and Critical Care Medicine (4 papers in 2 years) American Journal of Respiratory Cell and Molecular Biology (6 papers in 2 years)
1996-1998	Associate Editor, <u>American Journal of Respiratory Cell and Molecular Biology</u>
2000-2007	Editorial Board, <u>American Journal of Physiology:</u> <u>Lung Cellular and Molecular Physiology</u>
2002-2006	Editor, <u>Murray and Nadel's Textbook of Respiratory Medicine</u> 4 th Edition
2007-now	Editor, <u>Murray and Nadel's Textbook of Respiratory Medicine</u> 5 th Edition
2005-now	Editor, Website for <u>Murray and Nadel's Textbook of Respiratory Medicine</u> <u>www.respmedtext.com</u>
2010-2013	Editorial Board, <u>Apoptosis</u>

INVITED PRESENTATIONS**INTERNATIONAL**

International Chemokine Symposium, Bath, England, 1995 (Invited speaker)

International Meeting on the Toxicology of Natural and Man-

Made Fibrous and Non-Fibrous Particles, Lake Placid, NY, 1996 (Platform)

International Meeting of the Formosan Medical Association, Taiwan, 1999 (Platform)

American Thoracic Society International Conference. 1986 (Plenary talk); 1988 (Invited speaker, Course on the Academic Pulmonary Physician); 1989 (Invited speaker, Forum on Training and Transition); 1992 (Co-Chair, Poster Symposium); 1993 & 1994 (Co-Chair, Poster Discussion Symposium, Symposium); 1994 (Invited Speaker, Symposium); 1995 (Invited Speaker, Symposium; Meet the Professor Seminar); 1995 & 1997 (Chair, Speaker, Mini-symposium); 1996 (Co-chair, Speaker, Minisymposium); 1997 (Featured speaker, Minisymposium); 1999 (Invited speaker); 2000 (Speaker); 2001 (Co-chair, speaker, Mini-symposium); 2003 (Chair, Symposium); 2004 (Co-chair, Symposium; Meet the Professor Seminar); 2005 (Invited plenary speaker); 2006 (Invited Speaker, Participant in Expert Clinician Panel); 2007 (Co-Chair, Symposium; Presenter, Trudeau Award; Participant in Master Clinician Panel); 2008 (Participant in Master Clinician Panel; Co-Chair Symposium; Presenter); 2009 (Invited Clinical Expert in Pleural Disease; Participant in Master Clinician Panel; Co-Chair, Minisymposium)

International Mesothelioma Interest Group. San Francisco, 1993 (Invited Speaker); Paris, 1995 (Co-organizer, speaker); United Kingdom, 1999 (Speaker); Brescia, Italy, 2003 (Co-organizer, Speaker); Chicago, 2006 (Organizer Apoptosis Satellite Session, Co-chair, speaker). 2008 (Invited Plenary Speaker, Co-chair of symposium), 2009 (Program Planning Executive Committee, Speaker, Chair Symposia)

NATIONAL

1988 Advances in Internal Medicine, San Francisco, CA
 1989 Meet the Professor, American College of Physicians, San Francisco, CA
 1989 University of Oklahoma Health Science Center, Oklahoma City, OK
 1989 St. Luke's-Roosevelt Hospital Center, Columbia University, New York, NY
 1990 National Institutes of Health Workshop, Bethesda, MD
 1990 Invited Speaker, Scientific Conference on Acute Lung Injury,
 American Heart Association, Dallas, TX
 1990 Advances in Internal Medicine, San Francisco, CA
 1991 Advances in Internal Medicine, San Francisco, CA
 1994 Chair and Invited Speaker, Cambridge Health Institute Conference on
 Inflammatory Cytokine Antagonists, Philadelphia, PA
 1994 Invited Speaker, University of Pennsylvania, Philadelphia, PA
 1994 Gordon Conference on Chemotactic Chemokines
 1994 University of Pennsylvania, Philadelphia, PA
 1995 Texas Thoracic Society, Austin, TX
 1996 Selected Participant, Professional Development Seminar for Senior
 Women in Medicine, Association of American Medical Colleges,
 Washington DC
 1996 Co-Moderator, Pleural Disease Minisymposium.
 American College of Chest Physicians, San Francisco, CA
 1997 Visiting Pulmonary Scholar, Duke-UNC-NCSU-NIEHS-EPA-CIIT, NC
 1998 Visiting Speaker, St. Thomas Hospital, Vanderbilt Univ, Nashville, TN
 1998 Invited Speaker, First Annual Symposium on the Pleura,
 St. Thomas Hospital, Vanderbilt, TN.

- 1998 Visiting Professor, Medical Grand Rounds, University of Texas at Tyler, TX.
- 2000 Visiting Professor, Stanford University, Palo Alto, CA
- 2000 Invited Participant and Co-Chair, American Thoracic Society-
National Institute of Environmental Health Science (ATS-NIEHS)
Workshop-Toronto, Canada
- 2002 Visiting Professor, University of Southern California, Los Angeles, CA
- 2002 Visiting Professor, Yale University, New Haven, CT
- 2003 Visiting Professor, University of Montana,
Center for Environmental Health Sciences, Missoula, MT
- 2004 Visiting Professor, University of Pennsylvania, Philadelphia, PA
- 2006 Co-Chair, Minisymposium, Experimental Biology, San Francisco, CA.
- 2006 Invited Participant and Co-Chair, International Mesothelioma Interest Group,
Apoptosis Satellite Symposium, Chicago, IL
- 2006 Invited Speaker, University of Chicago School of Medicine
- 2006 Visiting Professor, Northwestern University School of Medicine
- 2007 Invited Speaker, International Mesothelioma Program,
Harvard Medical School/Brigham & Women's Hospital, Boston, MA
- 2008 Invited Speaker, International Mesothelioma Interest Group, Amsterdam
- 2009 Invited Speaker, NIH Mesothelioma Symposium, Bethesda, MD.
- 2009 Invited Speaker, ATS/Hawaii Thoracic Society State of the Art Course,
Maui, HI
- 2009 Invited Outside Reader, PhD Dissertation, Bonnie Lau, Dept of Pathology,
Brown University, Providence, RI
- 2009 Invited Speaker, International Mesothelioma Program,
Workshop on Preclinical Drug and Target Discovery,
Brigham and Womens Hospital, Boston, MA
- 2010 Invited Speaker, Mesothelioma Applied Research Foundation,
Washington, DC.

REGIONAL AND OTHER INVITED PRESENTATIONS

- 1988 Medical Grand Rounds, SFGH
- 1993 Research Conference on Lung Injury, Genentech, So SF
- 1994 SFGH Cellular and Molecular Medicine Seminar
- 1995 Medical Grand Rounds, VAMC/ SFGH
- 1996 Medical Grand Rounds, Moffitt/UCSF
- 1996 Selected Participant, Senior Women's Conference,
University of California San Francisco
- 1996 Panel Discussant, UCSF Department of Medicine.
- 1996 Medical Grand Rounds, SFGH
- 1997 Invited Speaker, UCSF Pulmonary Retreat, Asilomar, CA.
- 1998 Invited Speaker, UCSF Pulmonary Retreat, Asilomar, CA.
- 1998 Invited Speaker, Thoracic Oncology Conference, UCSF/Stanford/Mt. Zion
- 2000 Medical Grand Rounds, Stanford University
- 2000 Invited speaker, Division of Pulmonary and Critical Care, Stanford Univ.
- 2000 Invited Speaker, Pulmonary Research Retreat, UCSF
- 2003 Medical Grand Rounds, SFGH
- 2003 Invited Speaker, Recent Advances in Pulmonary and Critical Care Medicine
- 2003 Invited Speaker, Pulmonary Research Retreat, UCSF
- 2005 Invited Speaker, Thoracic Oncology Research Group, UCSF
- 2005 Invited Speaker, Radiation Oncology Grand Rounds, UCSF and Mt Zion
- 2006 Invited Speaker, Thoracic Oncology Research Group, UCSF
- 2006 Invited Speaker, Pulmonary Research Retreat, UCSF
- 2008 Invited Speaker, Dean's Seminar Series, SFGH
- 2009 Invited Speaker, Pulmonary Research Retreat, UCSF

GOVERNMENT AND OTHER PROFESSIONAL SERVICE

1994	Ad Hoc Member, Comparative Medicine Review Committee, NIH
1999	Ad Hoc Reviewer, National Heart, Lung, and Blood Institute
2001	Ad Hoc Reviewer, National Cancer Institute
2002	Invited Member, NIH Center for Scientific Review, Special Emphasis Panel, Experimental Therapeutics Panel (ZRG1 ET-1).
2004	Site Reviewer, Program Project Grant Review Committee, NCI. MGH/Brigham and Women's, Boston, MA
2005	Invited Member, National Asbestos Research Working Group National Health and Medical Research Council, Australia
2006	Invited Participant, NHLBI Strategic Plan for the Division of Lung Diseases
2009	Reviewer, FY09 Peer-reviewed Medical Research Program, AIBS, US Army Medical Research and Materiel Command
2009-now	Expert Panel Member, NIEHS Asbestos Mechanism of Action Workshop

**UNIVERSITY AND PUBLIC SERVICE
UNIVERSITY SERVICE**UCSF CAMPUS-WIDE

1996-1998	Member, Chancellor's Award for the Advancement of Women Committee
2003-now	Member, Academic Senate Committee on Research, UCSF
2005	Chair, Academic Senate Task Force Reviewing University of California Policy on Human Subject Injury
2006-2007	Member, Reconvened SFGH Subcommittee of the Chancellor's Advisory Committee on the Long Range Planning Amendment
2006 & 2007	Faculty Presenter, Inaugural Faculty Welcoming Week, Chancellor's Council on Faculty Life, "Building a Research Career"
2007	Small group leader, Junior faculty retreat, Striving & Thriving in the Academic World, Chancellor's Advisory Committee on the Status of Women

SCHOOL OF MEDICINE

1988-1989	Internship Selection Committee, Univ. of Calif, San Francisco
2005	Member, Stewardship Review Committee, Chair of Dermatology, UCSF
2005	Member, Ad Hoc Subcommittee on Faculty Misconduct, Office of Academic Affairs
2006- 2007	Member, Search Committee for Chair, Department of Medicine
2006- 2007	Member, Select Subcommittee of Search Committee for Chair, Department of Medicine, UCSF
2007	Invited speaker, The Senior Faculty Career Challenge, Dean's Office of School of Medicine. "Strategies for revitalizing divisional goals/function"
2007- now	Member, Scholarships and Awards Committee, UCSF School of Medicine

DEPARTMENT OF MEDICINE

1988-1989	Member, Bylaws Committee, San Francisco General Hospital
1989	Member, Program Planning Committee Recent Advances in Pulmonary & Critical Care Medicine (8th Annual), University of California, San Francisco
1989-2000	Member, Critical Care Committee, San Francisco General Hospital
1990-1991	Chair, Program Planning Recent Advances in Pulmonary & Critical Care Medicine (9th and 10th Annual), Univ of California, San Francisco

1990-present	Member, Steering Committee of the Pulmonary Research Group, University of California, San Francisco (elected)
1993-present	Member, Pulmonary Fellowship Selection Committee, University of California, San Francisco
1994-present	Member, Pulmonary Research Group Retreat Planning Committee
1994-1996	Member, Search Committees for Chief of Surgical Research and Chief of Rheumatology, San Francisco General Hospital
1996-1998	Member, Task Force on Diversity, University of California, SF
1996-1999	Member, Search Committee for Joint Appointment in Radiology/Pulmonary and Critical Care Medicine, San Francisco General Hospital
1996-2002	Member, Promotions Subcommittee, Academic Senate, University of California, San Francisco
1996-1997	Member, Executive Committee, Department of Medicine, SFGH
1996-2002	Board Member, UCSF/Macy's Center for Creative Therapies, San Francisco General Hospital
1997	Member, Search Committee, first Associate Chair for Biomedical Research, Department of Medicine, UCSF
1997-1998	Member, Search Committee for Chief of Cardiology, SFGH
1998-1999	Member, Search Committee for Chief of Gastroenterology, SFGH
1998-1999	Member, Search Committee, Chief, Pulmonary and Critical Care Med, UCSF
1998-2000	Member, General Clinical Research Center Advisory Committee
1999-2000	Member, Search Committee for Thoracic Surgeon, SFGH
2000	Member, Search Committee for Chief of Radiology, SFGH
2000	Member, Search Committee for Manager, Department of Medicine, SFGH
2003-2004	Chair, Search Committee Chief of Pulmonary & Critical Care Medicine, Veteran's Administration Medical Center, UCSF
2005-2006	Member, Search Committee for Chest Radiologist, SFGH
2005	Member, John Carbone Chair Nominating Committee
2006-	Member, Search Committee for Scientist, Surgical Research Laboratory
2006-2007	Member, Search Committees for Gastroenterology FTE
2006-current	Chair, Committee to Establish John F. Murray Distinguished Professorship
2008-now	Member, Search Committee for Lung Biology Center Physician-Scientist
2008-now	Member, Search Committee for Chief, Division of Pulmonary & Critical Care Medicine, UCSF
2009-now	Chair, Search Committee for Pulmonary Faculty Member, SFGH/UCSF.
2009-now	Member, Recruitment and Retention Workgroup, Department of Medicine Strategic Plan

PUBLIC SERVICE

1997	Presenter on Asbestos-Related Diseases, Gloria R. Davis Academic Middle School Asbestos Exposure & Risk Assessment, S.F. Department of Public Health
1997	Coordinator, Cigarette Smoke Demonstration, Take Your Daughters to Work Day
2005-7	Lecturer on Avian and Mammal Lungs, San Francisco Day School
2008-9	Fundraiser, Annual Fund, International High School, San Francisco.
2009-now	Class Captain, Annual Fund, International High School, San Francisco.

TEACHING AND MENTORINGOther Courses

1990-now	Pulmonary Physiology Seminars (2 hours/year)
1990	Faculty Leader, Preparing Interns for Residency
1990-now	Medical Service Conferences
1991	Women's Medical Student Association Retreat
1993-2003	Lecturer to Medical Residents (Parnassus, SFGH, VAMC)
1987-2002	Speaker, Recent Advances in Pulmonary & Critical Care Medicine
1998-now	Summer Seminar Series and Practical Sessions on Pleural Disease
2004	Faculty Coach and Presenter for Junior Faculty, Mid-Term Appraisal for Faculty at UCSF
2004	Annual PIBS/BMS Course, Ethics and the Responsible Conduct of Research
2005	Lecturer to Medical Residents (Parnassus, SFGH, VAMC)
2005-2007	Workshop Leader, Mid-term Appraisal for Faculty at UCSF

Predoctoral Students Supervised

<i>Dates</i>	<i>Name</i>	<i>Position while supervised</i>	<i>Current position</i>
1995-1997	Sudha Rani Narasimha	Medical Student	Medical Resident, UCLA
1997-1999	Jack Wu	Undergraduate	Medical resident Cook County, Chicago, IL
2002-2004	Kevin Lee	Undergraduate	Medical student
2009- now	Nikita Kolhatkar	Graduate	current

Graduate Students Supervised

<i>Dates</i>	<i>Name</i>	<i>Position while supervised</i>	<i>Current position</i>
2005-now	Dario Barbone	Graduate student	Postdoctoral scholar, UCSF
2008-9	Bonnie Lau	Graduate student	MD/PhD Brown University

Postdoctoral Fellows Supervised

<i>Dates</i>	<i>Name</i>	<i>Position while supervised</i>	<i>Current position</i>
1989-1991	Alice M. Boylan, MD	Pulmonary research fellow	Associate Professor of Medicine Medical Univ of South Carolina
1991-1993	Rex Yung, MD	Pulmonary research fellow	Associate Professor of Medicine Johns Hopkins Univ.
1993-1995	Hans Folkesson, PhD	Postdoctoral research fellow	Associate Professor of Physiology Northeastern Ohio University, OH
1995-1996	Jamie Bigelow, MD	Pulmonary research fellow	Pulmonologist St. Francis Hospital, SF
1996-1997	Evaldo Marchi, MD	Visiting research fellow	Professor of Surgery Sao Paulo, Brazil
1998-2000	Tom Geiser, MD	Postdoctoral research fellow	Associate Professor of Medicine University Hospital, Bern, SW
2000-2001	Masa Ishigaki, MD PhD	Visiting research fellow	Associate Professor, Japan
2000-2003	Claire Vivo, PhD	Postdoctoral research fellow	Research Scientist Ordway Research Institute, NY
2003-2004	Ki Up Kim, MD	Visiting research fellow	Associate Professor of Medicine Soonchunhyang University Hospital South Korea
2004-2005	Lorriana Leard, MD	Pulmonary research fellow	Assistant Prof of Medicine, UCSF
2003-2006	Keith Abayasiriwardana, PhD	Postdoctoral research fellow	Research Scientist, Merck Laboratories, London, UK

2006- 2008	Tsung-Ming Yang, MD	Postdoctoral research fellow	Assistant Professor, Chiayi Chung-Gung Memorial Hosp Chiayi, Taiwan
2008-on	Eunice Kim, MD	Pulmonary Fellow	Pulmonary Research Fellow
2008-on	Denitza Blagev, MD	Pulmonary Fellow	Pulmonary Research Fellow
2008-on	Joyce Lee, MD	Pulmonary Fellow	Pulmonary Research Fellow
2009-on	Joshua Galanter, MD	Pulmonary Fellow	Pulmonary Research Fellow

INFORMAL TEACHING

1987-now: Attending Rounds, Pulmonary Consult Service, SFGH
(1 month/year with 2 fellows, 1 medical student and/or resident)

1987-now: Attending Rounds, Medical ICU Service, SFGH
(1.5 month/year with 4 3rd year residents and 4 interns/ informal and formal teaching)

1999-2002 Attending Rounds, Medicine Service, SFGH

FACULTY MENTORING

I have selected some representative examples of mentoring relationships from recent years.

<i>Dates</i>	<i>Name</i>	<i>Position While Mentoring</i>	<i>Role</i>	<i>Current Position</i>
2000-2002	David Morris, MD	Division chief	Informal advisor	Head Respiratory Research Roche, Palo Alto, CA
2000-2005	Robert Jasmer, MD	Division chief	Advisor	Physician, private practice
2004-now	Payam Nahid, MD	Division chief	Advisor, Reviewed grants	Asst Prof, UCSF
2004-now	Mary Gray, MD	LBC Assoc Director	Recruited into LBC, collaborator/advisor	Assoc Prof, UCSF
2002-now	Laura Koth, MD	LBC Assoc. Dir.	Career advisor	Asst Prof, UCSF
2004-now	Lorriana Leard, MD	Lab head/Div.chief	Advisor, formal mentor	Asst Prof, UCSF
2005-2007	Dana McClintock, MD	Career committee	Selected formal mentor	Pulmonary fellow
2005-now	Harold Collard, MD	Division chief	Advisor, reviewed grants	Asst Prof, UCSF
2006-now	Janet Diaz, MD	Division chief	Advisor	Asst Prof, UCSF

TEACHING AWARDS AND NOMINATIONS:

1991 Distinction in Teaching Award, Academic Senate, UCSF

2003 Nomination for Most Outstanding Teacher, UCSF Women In Medicine

2007 & 2008 Nominations for Subspecialist Consultant of the Year Award,
SFGH Dept of Medicine

SUMMARY OF TEACHING HOURS:

2007-2008 Total anticipated hours of teaching: 540
Formal class or course teaching hours: 20
Informal teaching hours: 250
Mentoring hours: 270

2008-2009 Total anticipated hours of teaching: 520
Formal class or course teaching hours: 20
Informal teaching hours: 250
Mentoring hours: 250

2009-2010 Total anticipated hours of teaching: 540
Formal class or course teaching hours: 20
Informal teaching hours: 250
Mentoring hours: 270

RESEARCH AND CREATIVE ACTIVITIES

RESEARCH AWARDS AND GRANTS

ACTIVE:

Supporting Agency: Peer-Reviewed Medical Investigator-Initiated Research Program –
Department of Defense

Grant Title: *The Role of Macrophage-induced Inflammation in Mesothelioma.*

Role on project : Principal Investigator

Time Commitment to Project: 3 Calendar Months Effort (25%)

Total Funding Period: 04/01/2009 – 3/31/2012

Direct Dollars: \$300,000 / yr 1
\$900,000 / yr 1-3

Project Overlap: None

Goals: To demonstrate the functional significance of macrophages as promoters of tumor cell survival in mesothelioma and determine whether tumor-associated macrophages can be repolarized to enhance mesothelioma cell apoptosis.

Specific Aims: 1. To determine the functional significance of macrophage phenotype in mesothelioma. 2. To determine the functional significance of macrophages as regulators of mesothelioma apoptosis in vitro. 3. To define the functional significance of macrophage depletion or repolarization on mesothelioma survival in vivo.

Supporting Agency: Ireland- NCI Cancer Consortium

Total Funding Period: 1/1/2009 – 12/31/2011

Grant Title: *Role of the Bcl-2 Family in Apoptotic Resistance of Mesothelioma to Bortezomib.*

Role on project: Co-Principal Investigator

Time Commitment to Project: No time

Direct Dollars: \$135,000 / yr

Program Overlap: None

Goals: To support a post-doctoral fellow to determine the mechanism of apoptotic resistance in 3 dimensional models of mesothelioma.

Specific Aims: 1) To study the mitochondrial anti-apoptotic repertoire that determines resistance of mesothelioma to bortezomib. 2) To uncover the role of the mitochondrial anti-apoptotic repertoire that contributes to the acquired apoptotic resistance in 3D models to bortezomib.

Supporting Agency: NCI

Grant mechanism: RO1 CA135358-01

Total Funding Period: 8/11/2008 – 5/31/2012

Grant Title: *Targeted Liposomal Radiotherapy of Malignant Mesothelioma*

Role on project: Co-Investigator

Time Commitment to Project: 5%

Program Overlap: None

Goals: To engineer nanoliposomes as the dose delivery media to carry high payload of radionuclides for specific targeting of the mesothelioma tumor cells.

Specific Aims: 1) To identify optimal scFv antibodies targeting to malignant mesothelioma from a panel of 15 antibodies we have recently discovered. The native antibodies will be labeled with 99mTc for in vitro and in vivo. 2) To focus on the 177Lu labeling of the nanosized liposome conjugated with optimal antibody (177Lu-LP-scFv) to achieve high specific activity and characterize the targeting capability, stability and radiolysis in vitro 3) To determine the biodistribution and treatment efficacy of the best 177Lu-LP-scFv in mice with malignant peritoneal mesothelioma xenografts.

Past

NIH Institutional National Research Service Award (HL07185)	1983-1985
NIH Individual National Research Service Award (HL07271)	1985-1986
Academic Senate Committee on Research Grant	1986
Origin of pleural effusions in volume-loaded sheep	
Academic Senate Committee on Research Grant	1988
Origin of pleural effusions in hydrostatic pulmonary edema	
NHLBI Pulmonary Vascular SCOR (HL19155) (PI)	1986-1991
Dynamics of pleural liquid turnover in health and disease.	
American Lung Association Research Grant,	1987-1989
Formation of pleural effusions in pleural inflammation.	
Clinical Investigator Award, NHLBI (KO8 HL01893)	1987-1992
Comparative physiology of the normal and inflamed pleura.	
NHLBI Pulmonary Vascular SCOR (HL19155) (Co-Investigator)	1991-1993
Mechanisms of acute pleural and lung injury.	
Pretenure Award, University of California, San Francisco	1992-1993
Mechanisms of interaction of asbestos and mesothelial cells.	
Corvas International, San Diego, CA. (Co-Investigator)	1993-1994
The role of rabbit IL-8 in neutrophil-mediated inflammation.	
Genentech (PI)	1993-1994
The role of rabbit IL-8 in neutrophil-mediated inflammation.	
RO1 ES06331 (NIEHS) (PI)	1994-1998
Molecular interactions of asbestos and mesothelial cells.	
Genentech (PI)	1995-1997
The role of rabbit IL-8 in sepsis.	
Research Evaluation and Allocation Committee Grant, UCSF	1996-1997
The role of fiber internalization in mediating the toxic effects of asbestos on mesothelial cells.	
Principal Investigator, Tobacco-related Disease Research Program	1998-2001
Programmed cell death in cigarette-induced lung disease.	
RO1 ES08985 (NIEHS) (PI)	1997-2002
Protective role of apoptosis in asbestos pleural injury.	
RO1 ES08985 (NIEHS) (PI)	2000-2002
Supplement for microarray studies.	
Peterson Family Foundation (PI)	2005-2007
Role of Akt/mTOR in mesothelioma.	
Buzzi Foundation, Italy	2005-2007
Role of PI3K/Akt/mTOR pathway in resistance to apoptosis.	
RO1 CA95671 (NIH/NCI) (PI)	2003-2009
Amplification of TRAIL-induced apoptosis in mesothelioma.	
Mesothelioma Applied Research Foundation Award.	2007-2009
Antibody development against mesothelioma.	

PEER REVIEWED PUBLICATIONS

1. BROADDUS C, Dake M, Stulbarg MS, Blumenfeld W, Hadley K, Golden JA, Hopewell PC. Bronchoalveolar lavage and transbronchial biopsy for the diagnosis of pulmonary infections in patients with the acquired immunodeficiency syndrome. Ann Intern Med 102:747-752, 1985.
2. Wiener-Kronish JP, Goldstein R, Matthay RA, Biondi JW, BROADDUS VC, Chatterjee K, Matthay MA. Lack of association of pleural effusion with chronic pulmonary arterial and right atrial hypertension. Chest 92:967-970, 1987.
3. BROADDUS VC, Wiener-Kronish JP, Berthiaume Y, Staub NC. Removal of pleural liquid and protein by lymphatics in awake sheep. J Appl Physiol 64:384-390, 1988.
4. Berthiaume Y, BROADDUS VC, Gropper MA, Tanita T, Matthay MA. Alveolar liquid and protein clearance from normal dog lungs. J Appl Physiol 65:585-593, 1988.
5. Wiener-Kronish JP, BROADDUS VC, Albertine KH, Gropper MA, Matthay MA, Staub NC. Relationship of pleural effusions to increased permeability pulmonary edema in anesthetized sheep. J Clin Invest 82:1422-1429, 1988.
6. BROADDUS VC, Wiener-Kronish JP, Staub NC. Clearance of lung edema into the pleural space of volume-loaded anesthetized sheep. J Appl Physiol 68:2623-2630, 1990.
7. BROADDUS VC, Araya M, Carlton DP, Bland RD. Developmental changes in pleural liquid protein concentration in sheep. Am Rev Resp Dis 143:38-41, 1991.
8. Jacobson MA, Mills J, Rush J, Peiperl L, Seru V, Mohanty PK, Hopewell PC, Hadley WK, BROADDUS VC, Leoung G, Feigal DW. Morbidity and mortality of patients with AIDS and first-episode *Pneumocystis carinii* pneumonia unaffected by concomitant pulmonary cytomegalovirus infection. Am Rev Respir Dis 144:6-9, 1991.
9. BROADDUS VC, Araya M. Liquid and protein dynamics using a new, minimally invasive pleural catheter in rabbits. J Appl Physiol 72:851-857, 1992.
10. Boylan AM, Rüegg C, Hoeffel J, Kim KJ, Hébert CA, Pytela R, Sheppard D, Goldstein IM, BROADDUS VC. Evidence of a role for mesothelial cell-derived interleukin-8 in the pathogenesis of asbestos-induced pleurisy in rabbits. J Clin Invest 89:1257-1267, 1992.
11. BROADDUS VC, Hébert CA, Vitangcol RV, Hoeffel JM, Bernstein MS, Boylan AM. Interleukin-8 is a major neutrophil chemotactic factor in pleural liquid of patients with empyema. Am Rev Respir Dis 146:825-830, 1992.
12. BROADDUS VC, Feigal DW Jr. Starting an academic career: a survey of junior academic pulmonary physicians. Chest 105:1858-1863, 1994.
13. BROADDUS VC, Hoeffel JM, Boylan AM, Sadick M, Chuntharapai A, Kim KJ, Hébert CA. Neutralization of interleukin-8 inhibits neutrophil influx in a rabbit model of endotoxin-induced pleurisy. J Immunol 152:2960-2967, 1994.
14. Boylan AM, Hébert CA, Sadick M, Wong WL, Hoeffel JM, Hartiala KT, BROADDUS VC. Interleukin-8 is a major component of pleural liquid chemotactic activity in a rabbit model of endotoxin pleurisy. Am J Physiol Lung Cell Mol Physiol 267(11):L137-L144, 1994.

15. Folkesson HG, Matthay MA, Hébert CA, BROADDUS VC. Acid aspiration lung injury in rabbits is mediated by interleukin-8 dependent mechanisms. J Clin Invest 96:107-116, 1995.
16. Boylan AM, Sanan DA, Sheppard D, BROADDUS VC. Vitronectin enhances internalization of crocidolite asbestos by rabbit pleural mesothelial cells via the integrin $\alpha 5$. J Clin Invest 96:1987-2001, 1995.
17. BROADDUS VC, Yang L, Scavo LM, Ernst JD, Boylan AM. Asbestos induces apoptosis of human and rabbit pleural mesothelial cells via reactive oxygen species. J Clin Invest 98:2050-2059, 1996. (** identified by the Editors as being of broad interest*)
18. BROADDUS VC, Yang L, Scavo LM, Ernst JD, Boylan AM. Crocidolite asbestos induces apoptosis of pleural mesothelial cells: Role of reactive oxygen species and poly (ADP-ribosyl) polymerase. Environ Health Perspect 105 (Suppl 5):1147-1152, 1997.
19. Narasimhan SR, Yang L, Gerwin BI, BROADDUS VC. Resistance of pleural mesothelioma cell lines to apoptosis: relation to expression of Bcl-2 and Bax. Am J Physiol (Lung Cell Mol Physiol) 275(19): L165-L171, 1998.
20. Ernst JD, Yang L, BROADDUS VC. Preparation and characterization of an endogenously fluorescent annexin for detection of apoptotic cells. Anal Biochem 260:18-23, 1998.
21. Perkins RC, BROADDUS VC, Shetty S, Hamilton S, Idell S. Asbestos upregulates expression of the urokinase-type plasminogen activator receptor on mesothelial cells. Am J Respir Cell Mol Biol 21:637-646, 1999.
22. Miyazaki H, BROADDUS VC, Wiener-Kronish JP, Sawa T, Pittet J-F, Kravchenko V, Mathison JC, Nishizawa H, Hattori S, Yamakawa T, Yamada H, Kudoh I. The effects of two anti-inflammatory pretreatments on bacterial-induced lung injury. Anesthesiology 90:1650-1662, 1999.
23. Modelska K, Pittet J-F, Folkesson HG, BROADDUS VC, and Matthay MA. Acid-induced lung injury: protective effect of anti-interleukin-8 pretreatment on alveolar epithelial barrier function in rabbits. Am J Respir Crit Care Med 160:1450-1456, 1999.
24. Marchi E, Liu W, BROADDUS VC. Mesothelial cell apoptosis is confirmed in vivo by morphologic change in cytokeratin distribution. Am J Physiol Lung Cell Mol Physiol 278: L528-L535, 2000.
25. Levresse V, Renier A, Levy F, BROADDUS VC, Jaurand M-C. DNA breakage in asbestos-treated normal and transformed (TSV40) rat pleural mesothelial cells. Mutagenesis 15(3): 239-244, 2000.
26. Liu W, Ernst JD, BROADDUS VC. Phagocytosis of crocidolite asbestos induces oxidative stress, DNA damage and apoptosis in mesothelial cells. Am J Respir Cell Mol Biol 23(3): 371-378, 2000.
27. Wu J, Liu W, Koenig K, Idell SI, BROADDUS VC. Vitronectin adsorption to chrysotile asbestos increases phagocytosis and toxicity for mesothelial cells. Am J Physiol Lung Cell Mol Physiol 279:L916-L923, 2000.
28. Cambier S, Mu DZ, O'Connell D, Boylen K, Travis W, Liu W, BROADDUS VC, Nishimura SL. A role for the integrin $\alpha v \beta 8$ in the negative regulation of epithelial cell growth. Cancer Res 60(24): 7084-7093, 2000.
29. Liu W, Bodle E, Chen JY, Gao M, Rosen GD, BROADDUS VC. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and chemotherapy cooperate to induce apoptosis in mesothelioma cell lines. Am J Respir Cell Mol Biol 25(1):111-8, 2001.

30. Mu D, Cambier S, Fjellbirkeland L, Baron JL, Munger JS, Kawakatsu H, Sheppard D, BROADDUS VC, Nishimura SL. The integrin $\alpha\beta 8$ mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF- $\beta 1$. J Cell Biol 157(3):493-507, 2002.
31. Fjellbirkeland L, Cambier S, BROADDUS VC, Hill A, Brunetta P, Dolganov G, Jablons D, Nishimura SL. Integrin $\alpha\beta 8$ -mediated activation of TGF- β inhibits human airway epithelial proliferation in intact bronchial tissue. Am J Pathol 163(2):533-542, 2003.
32. Vivo C, Liu WH, BROADDUS VC. C-Jun N-terminal kinase contributes to apoptotic synergy induced by TRAIL plus DNA damage in chemoresistant, p53 inactive mesothelioma cells. J Biol Chem 278(28):25461-7, 2003.
33. Geiser T, Ishigaki M, van Leer C, Matthay MA, BROADDUS VC. H₂O₂ inhibits alveolar epithelial wound repair in vitro by induction of apoptosis. Am J Physiol Lung Cell Mol Physiol 287(2):L448-53, 2004.
34. BROADDUS VC, Dansen TB, Abayasiriwardana KS, Wilson SM, Finch AF, Swigart LB, Hunt AE, Evan GI. Bid mediates apoptotic synergy between TNF-related apoptosis-inducing ligand (TRAIL) and DNA damage. J Biol Chem 280:12486-12493, 2005.
35. Kim KU, Wilson SM, Abayasiriwardana K, Collins R, Fjellbirkeland L, Xu Z, Jablons DM, Nishimura SL, BROADDUS VC. A novel in vitro model of human mesothelioma for studying tumor biology and apoptotic resistance. Am J Respir Cell Mol Biol 33(6):541-8, 2005.
36. Acencio MM, Vargas FS, Marchi E, Carnevale GG, Teixeira LR, Antonangelo L, BROADDUS VC. Pleural mesothelial cells mediate inflammatory and profibrotic responses in talc-induced pleurodesis. Lung 185 (6):343-348, 2007.
37. Araya J, Cambier S, Markovics JA, Wolters P, Jablons D, Hill A, Finkbeiner W, Jones K, BROADDUS VC, Sheppard D, Barczak A, Xiao Y, Erle DJ, Nishimura SL. Squamous metaplasia amplifies pathologic epithelial-mesenchymal interactions in COPD. J Clin Invest 117 (11): 3551-3562, 2007.
38. Pespeni MH, Hodnett M, Abayasiriwardana KS, Roux J, Howard M, BROADDUS VC*, Pittet JF*. Sensitization of mesothelioma cells to TRAIL-induced apoptosis by heat stress via the inhibition of the 3-phosphoinositide-dependent kinase 1/Akt pathway. Cancer Res 67(6):2865-2871, 2007.

(* equal contributors as senior author)
39. Abayasiriwardana KS, Barbone D, Kim KU, Vivo C, Lee KK, Dansen TB, Hunt AE, Evan GI, BROADDUS VC. Malignant mesothelioma cells are rapidly sensitized to TRAIL-induced apoptosis by low dose anisomycin via Bim. Mol Cancer Ther 6(10):2766-2776, 2007.
40. Hassan R, BROADDUS VC, Wilson S, Liewehr DJ, Zhang J. Anti-mesothelin immunotoxin SS1P in combination with gemcitabine results in increased activity against mesothelin-expressing tumor xenografts. Clin Cancer Res 13(23):7166-7171, 2007.
41. An F, Drummond DC, Wilson S, Kirpotin DB, Nishimura SL, BROADDUS VC, Liu B. Targeted drug delivery to mesothelioma cells using functionally selected internalizing human single chain antibodies. Mol Cancer Ther 7(3):569-78, 2008.
42. Barbone D, Yang TM, Morgan JR, Gaudino G, BROADDUS VC. Mammalian target of rapamycin contributes to the acquired apoptotic resistance of human mesothelioma multicellular spheroids. J Biol Chem 283(19):13021-13030, 2008.

43. Wilson SM, Barbone D, Yang TM, Jablons DM, Bueno R, Sugarbaker DJ, Nishimura S, Gordon GJ, BROADDUS VC. mTOR mediates survival signals in malignant mesothelioma grown as tumor fragment spheroids. Am J Respir Cell Mol Biol 39(5):576-583, 2008.
44. Bidlingmaier S, He J, Wang Y, An F, Feng J, Barbone D, Gao D, Franc B, BROADDUS VC, Liu B. Identification of MCAM/CD146 as the target antigen of a human monoclonal antibody that recognizes both epithelioid and sarcomatoid types of mesothelioma. Cancer Res 69(4); 1570-1577, 2009.
45. Yang TM, Barbone D, Fennell DA, BROADDUS VC. Bcl-2 family proteins contribute to apoptotic resistance in lung cancer multicellular spheroids. Am J Respir Cell Mol Biol 41(1):14-23, 2009.

(* With accompanying editorial)

SUBMITTED

Chacko AD, Barbone D, Crawford N, Johnston PG, Cilli M, Piccardi F, Bertino P, Vialet J, Mutti L, BROADDUS VC, Gaudino G, Fennell DA. Reversal of bortezomib resistance in mesothelioma by a BH3 peptidomimetic targeting MCL-1/A1. (Submitted)

EDITORIALS

1. BROADDUS VC, Light RW. What is the origin of pleural transudates and exudates? Chest 102:658-659, 1992.
2. BROADDUS VC. Asbestos, the mesothelial cell and malignancy: a matter of life or death. Am J Respir Cell Mol Biol 17:657-659, 1997.
3. BROADDUS VC. Apoptosis and asbestos-induced disease – is there a connection? J Lab Clin Med 137(5):314-5, 2001.
4. BROADDUS VC. Diuresis and transudative effusions-changing the rules of the game. Am J Med 110(9):732-5, 2001.

WORKSHOP OR MEETING SUMMARIES

1. Crapo JD, BROADDUS VC, Brody AR, Malindzak G, Samet J, Wright JR; American Thoracic Society. ATS-NIEHS Workshop on lung disease and the environment; Where do we go from here? Am J Respir Crit Care Med 168(2):250-4, 2003.
2. BROADDUS VC. Advances in Mesothelioma Research. Report of the IMIG meeting. International Pleural Newsletter 2 (3): 10-11, 2004.
3. Carbone M, Albelda SM, BROADDUS VC, Flores RM, Hillerdal G, Jaurand, M-C, Kjaerheim K, Pass HI, Robinson B, Tsao A. Meeting Review: 8th International Mesothelioma Interest Group Oncogene 26 (49): 6959-6967, 2007.
4. Boylan A, Broaddus VC. Pleural Disease. In: Respiratory Disease in America: An ATS Fact Book. Schraufnagel, DE Editor 2010..

REVIEWS

1. Staub NC, BROADDUS VC, Zylak C, Lai-Fook SJ, Light RW, Vinegar R, Gaensler EA, Sahn SA. Pathophysiology of the pleural space. Am Rev Respir Dis 134:820-821, 1986.
2. BROADDUS VC, Berthiaume Y, Biondi JW, Matthay MA. Hemodynamic management of the adult respiratory distress syndrome. J Intensive Care Med 2:190-213, 1987.

3. BROADDUS C and Staub NC. Pleural liquid & protein turnover in health & disease. Sem in Respir Med 9:7-12, 1987.
4. Wiener-Kronish JP, BROADDUS VC. Interrelationship of pleural and pulmonary interstitial liquid. Ann Rev Physiol 55:209-226, 1993.
5. Matthay MA, BROADDUS VC. Fluid and hemodynamic management in acute lung injury. Sem in Respir Med 15:271-288, 1994.
6. BROADDUS, V.C. Infections in the pleural space: An update on pathogenesis and management. Sem in Respir Crit Care Med 16:303-314, 1995.
7. Marchi E, BROADDUS VC. Mechanisms of pleural liquid formation in pleural inflammation. Curr Opinion in Pulmonary Med 3:305-309, 1997.
8. Nishimura SL, BROADDUS VC. Asbestos-induced pleural disease. Clinics in Chest Medicine 19 (2): 311-329, 1998.
9. Leard LE, BROADDUS VC. Mesothelial cell proliferation and apoptosis. Respirology 9: 292-299, 2004.
10. Mutti L, BROADDUS VC. Malignant mesothelioma as both a challenge and an opportunity. Oncogene 23:9155-9161, 2004.

CHAPTERS

1. BROADDUS VC, Wiener-Kronish JP. Pleural diseases. In: Annual Review of Pulmonary and Critical Care Medicine, 1986-1987. Philadelphia: Hanley & Belfus, 1986, 115-132.
2. BROADDUS VC, Wiener-Kronish JP. Pleural diseases. In: Annual Review of Pulmonary and Critical Care Medicine, 1987-1988. Philadelphia: Hanley & Belfus, 1987, 197-219.
3. Wiener-Kronish JP, BROADDUS VC. Pleural diseases. In: Annual Review of Pulmonary and Critical Care Medicine, 1988-1989. Philadelphia: Hanley & Belfus, 1989, 85-102.
4. Wiener-Kronish JP, Boylan AM, BROADDUS VC. Pleural diseases. In: Annual Review of Pulmonary and Critical Care Medicine, 1991-1992. Philadelphia: Hanley & Belfus, 1991, 93-106.
5. BROADDUS VC. Cardiac diseases. In: Pulmonary Manifestations of Systemic Disease. JF Murray, ed. New York: Marcel Dekker, Inc., 1991; 59:149-190.
6. Light RW, BROADDUS VC. Disorders of the pleura: general principles and diagnostic approach. In: Textbook of Respiratory Medicine. JF Murray, JA Nadel, eds. 2nd edition. Philadelphia: WB Saunders Co., 1994, 2145-2163.
7. BROADDUS VC. Mechanisms of Pleural Liquid Turnover in the Normal State. UptoDate in Pulmonary and Critical Care Medicine (CDROM), S.E. Weinberger, Editor, American Thoracic Society, 1996-2004.
8. BROADDUS VC. Mechanisms of Pleural Liquid Accumulation in Disease. UptoDate in Pulmonary and Critical Care Medicine (CDROM), S.E. Weinberger, Editor, American Thoracic Society, 1996-2004.
9. BROADDUS VC, Hébert, CA. The Role of IL-8 in Inflammatory Diseases. In: Chemoattractant Ligands and Their Receptors. R. Horuk, Editor. CRC Press: New York. 1996, pp. 1-28.

10. Bigelow JM, BROADDUS VC. Empyema and Lung Abscess. In: Pulmonary/Respiratory Therapy Secrets. P.E. Parsons, J.E. Heffner, Editors. Hanley & Belfus, Inc. Medical Publishers, Phila, PA. 1996, pp. 179-185.
11. BROADDUS VC, Hébert CA. Neutralization of IL-8 in *in Vivo* models of lung and pleural injury. In: Methods in Enzymology. Chemokines and Chemokine Receptors. R. Horuk, Editor. Academic Press. Orlando, FL. 1997; 288: 161-181.
12. BROADDUS VC, Light RW. Disorders of the Pleura: General Principles and Diagnostic Approach (Chapter 73). In: Textbook of Respiratory Medicine. JF Murray, JA Nadel, RJ Mason, HA Boushey, eds. 3rd edition. Philadelphia: WB Saunders Co., 2000; pp. 1995-2012.
13. Light RW, BROADDUS VC. Pleural Effusion. (Chapter 74) In: Textbook of Respiratory Medicine. JF Murray, JA Nadel, RJ Mason, HA Boushey, eds. 3rd edition. Philadelphia: WB Saunders Co., 2000; pp. 2013-2041.
14. Light RW, BROADDUS VC. Pneumothorax, Chylothorax, Hemothorax and Fibrothorax. (Chapter 75) In: Textbook of Respiratory Medicine. JF Murray, JA Nadel, RJ Mason, HA Boushey, eds. 3rd edition. Philadelphia: WB Saunders Co., 2000; pp. 2043-2066.
15. Light RW, BROADDUS VC. Tumors of the Pleura. (Chapter 76) In: Textbook of Respiratory Medicine. JF Murray, JA Nadel, RJ Mason, HA Boushey, eds. 3rd edition. Philadelphia: WB Saunders Co., 2000; pp. 2067-2078.
16. BROADDUS VC, Jaurand MC. Asbestos Fibers and the Biology of Mesothelial Cells. In: Mesothelioma. P Chahinian, BWS Robinson, ed. Gordon & Breach Science Publishers, Harwood Academic Publishers 2004.
17. Bigelow JM, BROADDUS VC. Empyema and Lung Abscess. In: Pulmonary/Respiratory Therapy Secrets. 2nd edition. P.E. Parsons, J.E. Heffner, Editors. Hanley & Belfus, Inc. Medical Publishers, Phila, PA. 2001.
18. BROADDUS VC. Transudative pleural effusions. In: Pleural Diseases. Loddenkemper R, Antony V, Editors. Eur Respir Monograph 2002; 22, pp. 157-176.
19. Nahid P, BROADDUS VC. Liquid and Protein Exchange. (Chapter 3) In: Pleural Diseases: an International Textbook. Light RW and Lee G.Y.C. Editors. Hodder Arnold, London, England 2003; pp. 35-44.
20. BROADDUS VC. Physiology. (Chapter 6) and Transudates (Chapter 27). In: Derrame Pleural (Translation: Pleural Effusion). Vargas FS, Teixeira LR, and Marchi E, Editors. Roca Publishers, Brazil, 2003; pp. 15-24 and 233-248.
21. BROADDUS VC, Light RW. Pleural effusion (Chapter 68). In: Textbook of Respiratory Disease. RJ Mason, VC Broadus, JF Murray, JA Nadel, eds. 4th edition. Philadelphia: Elsevier 2005; pp.1913-1960.
22. Boylan AM, BROADDUS VC. Tumors of the pleura (Chapter 70). In: Textbook of Respiratory Disease. RJ Mason, VC Broadus, JF Murray, JA Nadel, eds. 4th edition. Philadelphia: Elsevier 2005; pp. 1989-2009.
23. BROADDUS VC. Fluid and solute exchange in normal physiological states. In: Textbook of Pleural Diseases, 2nd Edition. Light RW, Lee YGC (eds) London: Hodder, Arnold, 2008:43-48.

24. Light RW, BROADDUS VC. Pleural Effusion. In: Murray Nadel Textbook of Respiratory Medicine. RJ Mason, VC Broaddus, JF Murray, JA Nadel, King TE, Martin T, Schraufnagel D, eds. 5th edition. Philadelphia: Elsevier., 2010 (in press).
25. BROADDUS VC, Robinson BWS. Pleural Tumors. In: Murray Nadel Textbook of Respiratory Medicine. RJ Mason, VC Broaddus, JF Murray, JA Nadel, King TE, Martin T, Schraufnagel DE, eds. 5th edition. Philadelphia: Elsevier, 2010 (in press).

RESEARCH PROGRAM

SIGNIFICANT PUBLICATIONS

BROADDUS VC, Dansen TB, Abayasiriwardana KS, Wilson SM, Finch AF, Swigart LB, Hunt AE, Evan GI. Bid mediates apoptotic synergy between TNF-related apoptosis-inducing ligand (TRAIL) and DNA damage. J Biol Chem 2005; 280:12486-12493.

We uncovered the molecular mechanism of apoptotic synergy between the damage-intrinsic pathway of apoptosis and the death receptor-extrinsic pathway, showing that the DNA damage pathway sensitized the mitochondria to the Bid signal generated by TRAIL. (I performed all of the experiments during my sabbatical in Gerard Evan's laboratory, directed the work under his guidance and wrote most of the paper. This work has led to ongoing collaboration and follow up studies).

For all the remaining publications performed in my own laboratory after my sabbatical, I had the original idea, directed all experiments, and wrote most of the paper.

Kim KU, Wilson SM, Abayasiriwardana K, Collins R, Fjellbirkeland L, Xu Z, Jablons DM, Nishimura SL, BROADDUS VC. A novel in vitro model of human mesothelioma for studying tumor biology and apoptotic resistance. Am J Respir Cell Mol Biol 2005; 33(6):541-8.

We developed a new ex vivo model designed to explore the apoptotic responses of actual human tumor. A role for the Akt/mTOR pathway was found in the apoptotic resistance of the human tumors. This has led to further collaborations and other papers submitted and in preparation.

Wilson SM, Barbone D, Yang TM, Jablons DM, Bueno R, Sugarbaker DJ, Nishimura S, Gordon GJ, BROADDUS VC. mTOR mediates survival signals in malignant mesothelioma grown as tumor fragment spheroids. Am J Respir Cell Mol Biol 39(5):576-583, 2008.

We advanced the human tumor ex vivo model first described in our previous study to study a particular survival pathway, Akt/mTOR, and showed that blockade of this pathway did sensitize certain tumors, but only those with high baseline activity of mTOR pathway. Such studies explain how biomarkers of mTOR activity could select patients for therapy with mTOR blockers.

Barbone D, Yang TM, Morgan JR, Gaudino G, BROADDUS VC. Mammalian target of rapamycin contributes to the acquired apoptotic resistance of human mesothelioma multicellular spheroids. J Biol Chem 283(19):13021-13030, 2008.

We developed an in vitro 3D model of tumor cells that demonstrated acquired multicellular resistance to apoptosis, and we uncovered one pathway that contributed to resistance, the mTOR pathway. Such studies have informed use of mTOR inhibitors in clinical trials.

Yang TM, Barbone D, Fennell DA, BROADDUS VC. Bcl-2 family proteins contribute to apoptotic resistance in lung cancer multicellular spheroids. Am J Respir Cell Mol Biol 2009 (Epub ahead of print) * **With accompanying editorial.**

We developed 3D spheroids in lung cancer and showed for the first time a role for the Bcl-2 family of proteins in the acquired multicellular apoptotic resistance to bortezomib, the proteasome inhibitor that currently has little activity in solid tumors. This points the way to the use of small molecule Bcl-2 inhibitors to enhance efficacy of bortezomib in lung cancer clinical trials.

RESEARCH PROGRAM**DESCRIPTION OF CURRENT RESEARCH**

My research program continues to focus on the apoptotic resistance of tumors, malignant mesothelioma and lung cancer. We have focused particularly on mesothelioma, as a highly refractory and chemoresistant tumor, and are applying our findings now to lung cancer. We are investigating the apoptotic signaling involved in bypassing resistance and inducing apoptosis, specifically by combining agonists of the two major apoptotic pathways: the death receptor pathway and the DNA damage/mitochondrial pathway. We have shown that, while each pathway alone fails to induce apoptosis in the tumor cells, the combination will induce synergistic apoptosis. We continue to explore this phenomenon, searching for non-toxic means of stimulating these pathways such as heat stress and JNK stimulators such as anisomycin.

We have incorporated 3-dimensional models in our study of resistance, both of the mesothelioma cell lines grown as multicellular spheroids and of the human mesothelioma tumor itself grown as tumor fragment spheroids. Both these models allow us to test the resistance in a more clinically relevant system. We have found that a major survival pathway, the PI3K/Akt/mTOR pathway, contributes to the resistance in these 3-dimensional structures. Ultimately however, we believe that the resistance is manifested at the mitochondria by an altered repertoire of anti- and pro-apoptotic molecules. In an ongoing collaboration with Dr. Dean Fennell of Belfast, No. Ireland, we are now exploring the mitochondria as a central integrator of apoptotic signaling.

As a further direction using 3-dimensional models, we are collaborating with Dr. Lisa Coussens in studying the interaction of tumor-associated macrophages with the tumor cells. We will use our models, 3D multicellular spheroids and human tumor fragments, to investigate the contribution of macrophages to the apoptotic resistance of the tumor cells. We have found that mesothelioma contains a high number of macrophages, far more than in lung cancer or other tumors; thus, if these macrophages can be eliminated or manipulated to change from a supportive role to an anti-tumor role, this could be of significant therapeutic benefit for this currently incurable tumor.

CURRICULUM VITAE

June 2010

Lisa M. Coussens, Ph.D.

Professor

Department of Pathology

University of California, San Francisco

513 Parnassus Ave, HSW450C

San Francisco, CA 94143-0502

Voice: (415) 502-6378

Fax: (415) 514-0878

Email: Lisa.Coussens@ucsf.edu

<http://cancer.ucsf.edu/coussens/index.php>**I. EDUCATION:**

1976 - 1980	San Francisco State University	B.A.	Biology
1988 - 1993	University of California, Los Angeles	Ph.D.	Biological Chemistry
1993 - 1997	University of California, San Francisco	Post-Doctoral Fellow	Cancer Biology

II. PRINCIPAL POSITIONS HELD:

1981 - 1988	Genentech, Inc., South San Francisco	Research Associate	Molec. & Devel. Biology
1997 - 1999	Univ. of California, San Francisco	Assistant Research Biochemist	Hormone Research Inst.
1999 - 2004	Univ. of California, San Francisco	Assistant Professor, In Residence	Cancer Research Inst. & Dept of Pathology
2004 - 2006	Univ. of California, San Francisco	Associate Professor, In Residence	Cancer Research Inst. & Dept. of Pathology
2006 - 2007	Univ. of California, San Francisco	Associate Professor	Dept. of Pathology & Cancer Research Inst.
2007 - present	Univ. of California, San Francisco	Professor	Dept. of Pathology & Cancer Research Inst.

OTHER POSITIONS HELD CONCURRENTLY:

1989 - 1992	Whittier College, Whittier, CA	Lecturer	Biology Dept
1992	Genentech, Inc., South San Francisco	Scientific Consultant	Dept. of Legal Affairs
2000-present	Helen Diller Family Comprehensive Cancer Center, UCSF	Co-Director	Mouse Pathology Core
2007-2009	<i>CANCER RESEARCH</i>	Deputy Editor	
2009-present	Helen Diller Family Comprehensive Cancer Center, UCSF	Co-Director	Program in <i>Cancer Immunity & Microenvironment</i>
2009-present	<i>CANCER RESEARCH</i>	Deputy Editor	<i>Breaking Advances</i> section

III. HONORS AND AWARDS:

1985	Recognition Award	Genentech, Inc.,
1986	Recognition Award	Genentech, Inc.,
1988	Recognition Award	Genentech, Inc.
2000 - 02	Hellman Family Award For Early Career Faculty	Univ. of Calif., San Francisco
2000 - 01	V Foundation Scholar	The V Fndt. for Cancer Research
2000 - 03	Edward Mallinckrodt, Jr. Fndt. Award for Medical Research	Edward Mallinckrodt, Jr. Fndt.
2002	Gertrude B. Elion Cancer Research Award	Am. Assoc. for Cancer Research
2006 - 11	Era of Hope Scholar Award	Department of Defense, Breast Cancer Research Program

IV. PROFESSIONAL ACTIVITIES

1999 - present	Member	Graduate Program in BioMedical Sciences (BMS)	Univ. of Calif., San Francisco
1999 - present	Member	Helen Diller Family Comprehensive Cancer Center	Univ. of Calif., San Francisco
2000 - present	Member	Graduate Program in Biological Sciences (PIBS)	Univ. of Calif., San Francisco
2001 - present	Co-Director	Mouse Pathology Core	Helen Diller Family Comprehensive Cancer Center, Univ. of Calif., San Francisco
2004 – present	Member	Program in Immunology	Univ. of Calif., San Francisco
2004 – 2007	Senior Editor	Cancer Research (Cell, Tumor and Stem Cell Biology Section)	American Association Cancer Research
2007 – 2009	Deputy Editor	Cancer Research (general)	American Association Cancer Research
2007 – 2009	Senior Editor	Cancer Research (Tumor Microenvironment Section)	American Association Cancer Research
2007 - 2010	Member	External Scientific Advisory Board, University of Minnesota Cancer Center	University of Minnesota Cancer Center
2007 – 2011	Member	External Scientific Advisory Board, CA U54: <i>Aging, Tumor Microenvironment and Prostate Cancer</i> ; P.I. Steve Plymate, Univ. of Washington, HMC.	University of Washington
2008 – 2011	Member	Board of Directors (<i>elected</i>)	American Association of Cancer Research
2009 - present	Member	External Scientific Advisory Board; Neuroblastoma Program Project Grant	Children's Hospital Los Angeles, Univ. of Southern California
2009 – 2011	Deputy Editor	Cancer Research (Breaking	American Association

Lisa M. Coussens, Ph.D.

2009 - present	Co-Director	<i>Advances section)</i> Program in <i>Cancer Immunity and Microenvironment</i>	Cancer Research Helen Diller Family Comprehensive Cancer Center, Univ. of Calif., San Francisco
2010-2012	Council Member	<i>Women in Cancer Research</i> , Council (elected)	American Association of Cancer Research
2010	Member	External Advisory Committee, P01, Program in <i>Motility and Invasion</i> , John Condeelis, Ph.D., PI, Program Director	Albert Einstein College of Medicine

Local, National and International Meetings Organized:

- 2005 Keystone Symposia, *Inflammation and Cancer*, Co-organizer with Dr. Ray DuBois, Vanderbilt Univ, TN), Breckinridge, CO, USA
- 2006 5th Annual Timberline Symp. on Epithelial Cell Biology, '*Intrinsic and Microenvironmental Regulation of Epithelial Cancer*', Co-Organizer with Dr. Harold Moses (Vanderbilt University, TN, USA), Timberline, OR, USA
- 2006 Co-Organizer (with Dr. Lewis Lanier), UCSF HDFCCC Annual Symposium, '*Inflammation & Cancer: Bench to Bedside*'.
- 2007 Keystone Symposia, *Inflammation and Cancer*, Co-Organizer with Drs. Fran Balkwill (Cancer Research UK) and Glenn Dranoff (Beth Israel Cancer Center, Harvard, MA); Santa Fe, New Mexico, USA
- 2008 AACR Special Conference: *Inflammation and Cancer*, Co-organizer with Drs. Michael Karin and Larry Marnett. Oahu, Hawaii, USA.
- 2008 International Society for Biological Therapy of Cancer (ISBTc), *2008 Workshop on Inflammation in Cancer Development*, Co-Organizer with Drs. Michael Karin, (UCSD), Steven Dubinett (UCLA), and George Weiner (WU); San Diego CA USA
- 2010 Co-Organizer (with Dr. Lewis Lanier), UCSF HDFCCC Program in *Cancer Immunity and Microenvironment* Symposium
- 2011 AACR Special Conference: *Tumor Microenvironments: New Therapeutic Opportunities*, Co-Organizer with Drs. Kornelia Polyak and Melody Swartz.

V. PROFESSIONAL ORGANIZATIONS**Memberships**

- 2000 - 2009 American Society for Matrix Biology
- 2000 - present American Association for Cancer Research
- 2001 – 2008 American Society for Cell Biology
- 2004 - present American Society for Investigative Pathology
- 2004 - 2009 International Protease Society

Service to Professional Organizations**American Association for Cancer Research**

- 2003 Subsection Co-chair (Tumor Progression, Invasion and Metastasis) Cellular, Molecular and Tumor Biology Subcommittee, AACR Program Committee for 94th Annual Meeting.
- 2003 Chair and organizer, Educational Session (Proteases: Successes and Failures): 94th Annual Meeting, Washington D.C., USA

Lisa M. Coussens, Ph.D.

- 2003 Minisymposium Co-chair (Inflammatory Mediators & Cancer): *94th Annual Meeting*, Washington D.C., USA
- 2004 - 2006 Member, Grants Committee
- 2005 Minisymposium Co-Chair (Inflammation, Microenvironment and Tumor Progression): *96th Annual Meeting*, Anaheim, CA USA
- 2005 Session Chair (Inflammation): *AACR Special Conference: Cancer, Proteases and the Microenvironment*, Bonita Springs, Florida. USA
- 2006 Subsection Co-chair (Tumor Progression, Invasion and Metastasis) of the Tumor Biology Subcommittee, AACR Program Committee for *97th Annual meeting*
- 2006 Minisymposium Co-Chair (Inflammation and Cancer): *97th Annual Meeting*, Washington DC, USA
- 2006 Co-Chairperson, Program Committee: *6th Annual Frontiers in Cancer Prevention Research Conference*, December 5-8, 2007, Philadelphia, PA USA.
- 2006-2009 Steering Committee Member: AACR Tumor Microenvironment Working Group (TME/AACR).
- 2007 Organizer, Education session (Inflammation and Cancer), *98th Annual Meeting*, Los Angeles, USA
- 2007 Minisymposium Co-Chair (Tumor Microenvironment): *98th Annual Meeting*, Los Angeles, CA USA
- 2007 Co-Chairperson, Program Committee: *2008 99th Annual Meeting of the AACR*. April 12-16, 2008, San Diego, CA. USA
- 2008 Program Committee Member, Tumor Microenvironment Subcommittee for *99th Annual Meeting of the AACR*. April 12-16, 2008, San Diego, CA. USA
- 2007-2010 Member, AACR Special Conferences Committee
- 2008 Co-Organizer Special Conference: *Inflammation and Cancer*, with Drs. Michael Karin and Larry Marnett. Oahu, Hawaii, USA.
- 2008-2011 Member, Board of Directors (elected)
- 2009 Member, 2009 Education Committee, 2009 100th AACR Annual Meeting, Denver, CO. USA
- 2009 Organizer and Chair: *Inflammation and Cancer: Novel Mechanisms Regulating Protumor Immunity* Major Symposium, 2009 100th AACR Annual Meeting, Denver, CO. USA
- 2009 Organizer and Chair: Education Session, *Aspects of the Tumor Microenvironment that Regulate Solid Tumor Development*, 2009 100th AACR Annual Meeting, Denver, CO. USA
- 2009 Co-Chairperson, Program Committee: *2010 101st Annual Meeting of the AACR*, April 17-21, 2010, Washington, DC USA
- 2009 Member, Scientific Review Committee for *Stand Up to Cancer Innovative Research Grants*
- 2009 Member, Selection Committee: *2010 Pezcoller Foundation-AACR International Award for Cancer Research*
- 2010-2013 Council Member, *Women in Cancer Research Council* (elected)
- 2010 Co-Chair, Minisymposium 'The Tumor Microenvironment and Therapeutic Strategies' *2010 101st Annual Meeting of the AACR*, April 17-21, 2010, Washington, DC USA

American Society for Cell Biology

- 2000 American Society for Cell Biology, photo credits in '*Exploring the Cell*' Ed. W. Wells
- 2001 Table Leader, Career Discussion Lunch, Women in Cell Biology and Education Committee, 40th Annual Meeting, Washington, DC, USA

Lisa M. Coussens, Ph.D.

- 2001 Co-chair and Co-organizer, Mini-symposium (Microenvironment/Extracellular Matrix in Development and Disease): 40th Annual Meeting, Washington, DC, USA
- 2003 Table Leader, Career Discussion Lunch, Women in Cell Biology and Education Committee of the ASCB, 42nd Annual Meeting, San Francisco, CA, USA
- 2006 Co-Chair Minisymposium (Cancer Mechanisms): 46th Annual Meeting, San Diego CA, USA

American Cancer Society

- 1999 14th Annual Excalibur Round Table, San Francisco, CA, USA
- 2000 San Mateo County Annual Volunteer Meeting, San Mateo, CA, USA

International Society for Preventive Oncology

- 2002 Session Chair (Chemoprevention): 6th Annual Meeting, Pasteur Institute, Paris, France.
- 2002 Poster Judge (Chemoprevention): 6th Annual Meeting, Pasteur Institute, Paris, France.

International Proteolysis Society

- 2007 Member, International Scientific Advisory Committee, 5th General Meeting of the International Proteolysis Society, Rion-Patras, GREECE.

International Society for Biological Therapy of Cancer

- 2008 Co-Organizer, 2008 Workshop on Inflammation in Cancer Development, San Diego CA, USA

Service to Professional Publications:

- 2003 - 2005 Associate Editor, **Cancer Research**
- 2005 – 2007 Editorial Board, **Carcinogenesis**
- 2004 – 2007 Senior Editor, **Cancer Research (Cell, Tumor and Stem Cell Biology Section)**
- 2007 – 2009 Senior Editor, **Cancer Research (Tumor Microenvironment Section)**
- 2007 – 2009 Deputy Editor, **Cancer Research**
- 2007 Guest Editor, PNAS Editorial Board
- 2008 Guest Editor (with Tyler Jacks), *Current Opinion in Genetics & Development*
- 2008 – present Editorial Board, **Cancer Microenvironment**
- 2009 – 2011 Deputy Editor for Breaking Advances, **Cancer Research**

Ad hoc reviewing

- 1994 Oncogene;
- 1995 Am J Pathology; Matrix Biology; J Cell Biology
- 1999 Am J Pathology; Cancer Letters; Nature Medicine; Nature; PNAS; Cell Motility & the Cytoskeleton; Cancer Research
- 2000 Am J Pathology; Cancer Research; Genes & Development; Int. J Cancer
- 2001 J Cell Biology; Int. J of Cancer; EMBO; Neoplasia; Cancer Research
- 2002 Cancer Research; Am J Pathology; Int. J Cancer; Biological Chemistry; Cancer Cell; Cancer Letters
- 2003 PNAS; Cancer Research; Int. J of Cancer; J Molecular Medicine; Biological Chemistry; Science; Cancer Cell; Nature Medicine; J Leukocyte Biology; Neoplasia; Am J Pathology
- 2004 Lancet; Cancer Cell; Cancer Research; American J Pathology; J Cell Biology; Nature Reviews Immunology; Nature Reviews Cancer; PNAS; J Biological Chemistry; Nature; J Exp Med; Int J Cancer
- 2005 Nature Medicine, Cancer Cell, Cancer Research; Am J Pathology; Cell; Nature; Nature Reviews Immunology; Nature Reviews Cancer; Carcinogenesis
- 2006 Nature Reviews Cancer; Nature; Nature Medicine; Cell; Cancer Research; Clinical Cancer

Lisa M. Coussens, Ph.D.

Research; J Exp Med; Cancer Cell; Am J Pathology; J Cell Biology
 2007 Cell; Nature; PNAS; J Cell Biology; Cancer Research; J Exp Med; Breast Cancer Research
 2008 Cancer Cell; PNAS; J Immunology; Nature; J Exp Med; Trends in Genetics; Current
 Opinions in Investigational Drugs
 2009 Cancer Cell; Cell; Nature; J Exp Med; J Clin Invest, Cancer Research; Int J Cancer;
 Oncogene, J Immunology
 2010 Nature, J Exp Med, Nature Medicine, J Invest Dermatology, Cell, Cancer Cell, J Clinical
 Onc; PNAS; J Clin Invest; Cancer Research; Dis Mech Models;

VI. INVITED PRESENTATIONS

Symposia and Workshops: International

- 1996 *Human Tumor Heterogeneity II: Cytometric Measurement of Growth Regulation and Genetic Alterations: International Society of Analytical Cytometry.* Kananaskas, Alberta, Canada.
- 1997 *GeneMedicine-Boehringer Mannheim Cancer Alliance: Technology Workshop.* Cancún Mexico.
- 2001 *2nd Annual International Protease Society.* Freising, Germany.
- 2002 *6th International Symposium on Predictive Oncology & Intervention Strategies,* Pasteur Institute, Paris, France
- 2002 **KEYNOTE LECTURE, Dutch Cancer Society Annual Symposium,** Luntern, The Netherlands
- 2002 **KEYNOTE LECTURE, Cancer: Genome, Signal & Environment, Takeda Genome Urology International,** Kyoto, Japan
- 2003 *2nd Annual International Symposium on Epithelial Biology,* Timberline, Oregon USA
- 2004 *10th International Congress of the Metastasis Research Society, 'Progress Against Tumor Progression',* Genoa Italy
- 2005 *2005 International Consortium Meeting of the Children's Tumor Foundation: Molecular Biology of NF1, NF2 and Schwannomatosis,* Aspen, CO, USA
- 2005 *International Symposium on Systems Genome Medicine - Bench to Bedside,* Institute of Medical Sciences University of Tokyo, Tokyo, Japan
- 2005 *Immunotherapy of Cancer, XI Annual Symposium of the Danish Cancer Society,* Copenhagen, Denmark
- 2005 *4th General Meeting of the International Proteolysis Society,* Quebec City, Canada
- 2006 *Centro Nacional de Investigaciones Oncológicas (CNIO) Cancer Conference: Inflammation and Cancer,* Madrid SPAIN
- 2006 *18th Annual Pezcoller Symposium 'Tumor Microenvironment: Heterotypic Interactions',* Trento ITALY
- 2006 *European Association for Cancer Research (EACR) 1st Annual Meeting,* Budapest HUNGARY
- 2006 *XXXIVth Meeting of the International Society for Oncodevelopmental Biology and Medicine (ISOBM: Tumor Biology, Detection and Therapy,* Pasadena, CA, USA
- 2006 *37th International Symposium of the Princess Takamatsu Cancer Research Fund 'Cancer Cells and Their Microenvironment',* Tokyo, JAPAN
- 2007 *4th International Conference on Tumor Microenvironment,* Florence, ITALY
- 2007 *2nd International Symposium on Cancer Metastasis and the Lymphovascular System: Basis for Rational Therapy,* San Francisco CA USA
- 2007 *CNIO – Nature Symposium on "Oncogenes and Human Cancer". The Next 25 Years",* Madrid SPAIN
- 2007 **KEYNOTE LECTURE, 7th International Symposium on Hodgkin Lymphoma,** Cologne,

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GERMANY

- 2007 **CANDLELIGHT LECTURE**, *Inflammation and Cancer: From molecular links to bed side*; Inaugural meeting for the *Istituto Clinico Humanitas*, Milan ITALY
- 2008 7th Annual International Congress on the Future of Breast Cancer, Kauai, Hawaii USA
- 2008 Cancer Research UK Cambridge Research Institute (CRI) Inaugural Annual Symposium, 'Unanswered Questions in the Tumour Microenvironment', Homerton College, Cambridge UK
- 2008 5th International Kloster Seeon Meeting, *Angiogenesis: Molecular Mechanisms and Functional Interactions*. Kloster Seeon, GERMANY
- 2008 **CANCER RESEARCH UK LECTURE**, NCRI Cancer Conference, Birmingham UNITED KINGDOM
- 2009 21ST Lorne Cancer Conference, Lorne AUSTRALIA
- 2009 6th International Symposium on the Intraductal Approach to Breast Cancer, Santa Monica CA USA
- 2009 **STATE-OR-THE-ART LECTURE**, International Cancer Conference, *CANCER 2009*, Dublin IRELAND
- 2009 19th Annual BioCity Symposium, 'Tumor Microenvironment in Cancer Progression', Tirku FINLAND
- 2009 **KEYNOTE LECTURE**, European Association of Cancer Research, Special Conference on *Inflammation and Cancer*, Berlin GERMANY
- 2009 7th International Symposium on Minimal Residual Cancer, Athens, GREECE
- 2009 Tri-Society Annual Conference of the Society for Leukocyte Biology, International Cytokine Society, and the International Society for Interferon and Cytokine Research, Lisbon, Portugal
- 2009 5th International Conference on Tumor Microenvironment, Versailles, FRANCE
- 2009 **PRESIDENT'S PLENARY SESSION**: Italian Cancer Society Annual Meeting, Milano ITALY
- 2010 **PLENARY LECTURE**, CHUV Research Day, University hospital (CHUV) and the Faculty of Biology and Medicine, Lausanne, SWITZERLAND.

UPCOMING INVITATIONS

- 2010 *NATURE - CNIO Cancer Symposium on Frontiers in Tumour Progression*, Madrid SPAIN
- 2011 41st Australian Society for Immunology (ASI), Adelaide, South AUSTRALIA

Symposia and Workshops: National

- 1994 *Current Transgenic Technology*, B & K Universal, San Mateo, CA, USA
- 1997 *Biology of Proteolysis*, Cold Spring Harbor Laboratory, NY, USA
- 1997 *Molecular Biology & Pathology of Neoplasia*, AACR, Keystone, CO, USA
- 1997 *Matrix Metalloproteinases*, Gordon Research Conference, Proctor Academy, New London, NH, USA
- 1998 *Proteolysis*, Gordon Research Conference, Colby-Sawyer College, New London, NH, USA
- 1998 *Cellular Targets of Viral Carcinogenesis*, AACR Special Conference. Dana Point, CA, USA
- 1998 *Mechanisms of Tumor Growth & Invasion Mediated by Proteolysis*, UCSF-Molecular Design Institute. San Francisco, CA, USA
- 1999 *Tumor Microenvironment*, Education Session, AACR Annual Meeting. Philadelphia, PA, USA
- 1999 *Matrix Metalloproteinases*, Gordon Research Conference, Colby-Sawyer New London, NH, USA.
- 2000 *Epithelial-Stromal Interactions & Tumor Progression Workshop*, National Cancer Inst., Bethesda, MD, USA
- 2000 10th National Conference of the Inflammation Research Association, Hot Springs, VA,

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USA

- 2001 *'Meet-the-Expert' Sunrise Session*, AACR Annual Meeting, New Orleans, LA, USA
- 2002 *Chemotherapy of Experimental & Clinical Cancer*, Gordon Research Conference, Colby Sawyer College, New London, NH, USA
- 2002 *Proteolytic Enzymes & their Inhibitors*, Gordon Research Conference, Colby Sawyer, New London, NH, USA
- 2002 *From the Cancer Cell to a Tumor - Tumors as Outlaw Organs*, Schilling Research Conference, The American Cancer Society, Aptos CA, USA
- 2002 *Cancer Intervention 2002*, Van Andel Research Institute, Grand Rapids, Michigan USA
- 2002 *Pathobiochemistry B Study Section Workshop*, Natl. Cancer Institute, Hilton Head, SC, USA
- 2002 *Proteases, Extracellular Matrix and Cancer*, AACR Special Conference, Hilton Head Island, SC, USA
- 2002 *ECM and Cancer*, Minisymposium, ASCB Annual Meeting, San Francisco, CA, USA
- 2003 *Matrix Metalloproteinases*, Gordon Research Conference, Big Sky, Montana, USA
- 2003 *Angiogenesis & Microcirculation*, Gordon Research Conference, Salve Regina, Newport R.I., USA
- 2003 *Inflammatory Cells and Cancer*, Symposium, American Society of Hematology 2003 Annual Meeting, San Diego, CA, USA
- 2003 *Validation of a Causal Relationship: Criteria to Establish Etiology*, National Cancer Institute, Cancer Etiology Branch, Washington, DC, USA.
- 2003 *Functional Imaging of Proteolysis*, Special Session, ASCB Annual Meeting, San Francisco, CA, USA
- 2004 Scleroderma Research Foundation Annual Scientific Workshop, San Francisco, CA, USA
- 2004 *Systems Biology of Cancer: The Tumor as an Organ*, Symposium, 95th AACR Annual Meeting. Orlando, FL, USA
- 2004 *Inflammation and Cancer*, Symposium, 95th AACR Annual Meeting. Orlando, FL, USA
- 2004 *Remarkable Role of the Microenvironment in Development and Disease Pathogenesis*, Symposium; Experimental Biology 2004, Sponsored by: the Assoc. of Anatomy, Cell Biology and Neurobiology, Washington, D.C., USA.
- 2004 *Molecular and Cellular Basis of Disease: Structure and Function of the Extracellular Matrix in Disease: Novel Roles and Regulation of MMPs and TIMPs in Disease*, Symposium; Experimental Biology 2004, Sponsored by: the Am. Society of Investigative Pathology, the American Society for Matrix Biology and the North American Vascular Biology organization. Washington, D.C., USA.
- 2004 Pacific Coast Protease Workshop, Half Moon Bay, CA, USA.
- 2004 19th Aspen Cancer Conference: *Mechanisms of Toxicity, Carcinogenesis, Cancer Prevention and Cancer Therapy*. Aspen, CO, USA.
- 2005 Keystone Symposia, *The Role of Microenvironment in Tumor Induction and Progression (J5)*, Banff, Alberta CANADA
- 2005 Keystone Symposia, *Inflammation and Cancer (B8)*, Breckenridge, CO, USA
- 2005 *Symposium on Inflammation, Repair and Carcinogenesis in Liver, Pancreas and Colon*. UCSF Liver Center and the Program in Gastrointestinal Cancer of the UCSF Cancer Center, Rohnert Park, CA, USA
- 2005 *In the Forefront of Advances in Cancer Research*, Symposium, 96th AACR Annual Meeting. Anaheim, CA, USA
- 2005 *Macrophage Symposium*, AMGEN, Seattle, WA, USA
- 2005 *Immune Response to Cancer Symposium*, 41st Annual Meeting, American Society Clinical Oncology (ASCO), Orlando. FL. USA
- 2005 *Phagocyte*, Gordon Research Conference, New London, CT, USA

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- 2005 *Mouse Models of Human Cancer Consortium*, Annual Steering Committee Meeting, New Brunswick, NJ USA
- 2005 *Matrix Metalloproteinases*, Gordon Research Conference, Big Sky, Montana, USA
- 2005 *Annual Buffalo Regional Conference on Immunology*, Buffalo, NY, USA
- 2005 2005 Montagna Symposium on 'Tissue repair - molecular mechanisms and clinical challenges', Salishan Lodge, OR, USA
- 2005 4th Annual AACR Conference on *Frontiers in Cancer Prevention Research*, Baltimore MD, USA
- 2005 AACR Special Conference, *Cancer, Proteases and the Microenvironment*, Bonita Springs, Florida. USA
- 2006 Timberline Annual Symposium on Epithelial Biology, *Intrinsic and Microenvironmental Regulation of Epithelial Cancer*, Timberline Lodge, Oregon, USA
- 2006 Keystone Symposium, *Molecular Targets for Cancer Prevention*, Granlibakken Resort, Tahoe City, CA, USA
- 2006 *Inflammation and Cancer*, Symposium, 97th AACR Annual Meeting. Washington, D.C., USA
- 2006 Lineberger Cancer Center's 30th Annual Scientific Symposium, University of North Carolina, Chapel Hill, North Carolina, USA
- 2006 KEYNOTE LECTURE, *Vanderbilt-Ingram Cancer Center Retreat 2006*, Vanderbilt University, Nashville TN, USA
- 2006 **TUMOR BIOLOGY PLENARY LECTURE**, *Advances in Neuroblastoma Research 2006*, Los Angeles, CA, USA
- 2006 *Genetic, Cellular and Microenvironmental Determinants of Tumor Progression and Metastasis: A 'TPM' Workshop Honoring Martin L Padarathsingh, Ph.D.* TPM Study Section Workshop, Natl. Cancer Institute, Georgetown, VA, USA
- 2006 ASCO/Federation of European Societies Symposium: *Inflammation in Cancer Progression*, 2006 ASCO Annual Meeting, Atlanta, GA, USA
- 2006 AACR Special Conference, *Mouse Models of Cancer*, Cambridge, MA, USA
- 2006 AACR Special Conference, *Tumor Immunology: An Integrated Perspective*. Miami, FL, USA
- 2007 7th AACR-Japanese Cancer Association Joint Conference: *In the Forefront of Basic and Translational Cancer Research*, Waikoloa, Hawaii, USA
- 2007 Keystone Symposium, *'Mouse Models at the Frontiers of Cancer Discovery'*, Whistler, British Columbia, CANADA
- 2007 Keystone Symposium *'Inflammation and Cancer'*, Santa Fe, NM, USA
- 2007 AAAS Annual Meeting, *Healthy Aging: Inflammation and Chronic Diseases* Symposium, San Francisco, CA USA
- 2007 Tumor Microenvironment and Tumor-Stromal Interactions Workshop: Sponsored by Biogen Idec Inc., Oncology Discovery Research, San Diego CA USA
- 2007 American Thoracic Society 2007 International Conference, *San Francisco Science: Inflammation, Immunity and Signaling*. San Francisco, CA USA
- 2007 22nd Aspen Cancer Conference: Mechanisms of Toxicity, Carcinogenesis, Cancer Prevention and Cancer Therapy, Aspen CO, USA
- 2007 Gordon Research Conference, *Epithelial Differentiation & Keratinization*, Bryant University, Smithfield, RI, USA
- 2007 AACR, *Frontiers in Cancer Prevention Research Conference*, Philadelphia, PA, USA
- 2007 National Cancer Institute Workshop, *'Profiling of Immune Response to Guide Cancer Diagnosis, Prognosis and Prediction of Therapy'*, Bethesda, MD, USA
- 2008 47th Midwinter Conference of Immunologists, *'Meeting the challenge: Immunobiology in health and disease'*, Asilomar, CA USA

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- 2008 AACR-TREC-NCI Conference on *Energy Balance and Cancer: Mediators and Mechanisms*, Lansdowne, VA USA
- 2008 Keystone Joint Symposium, 'Cell Death in the Immune System / Cell Death and Cellular Senescence', Beaver Run Resort in Breckenridge, CO, USA
- 2008 Keystone Symposium, 'Inflammation, Microenvironment and Cancer', Snowbird Resort in Snowbird, Utah, USA
- 2008 The John F. Anderson Memorial Lectures in Medicine, 'The Linkage between Inflammation and Cancer', University of Virginia, Charlottesville VA, USA
- 2008 *Tumor Microenvironment Symposium*, Stony Brook University, Stony Brook. NY. USA
- 2008 **KEYNOTE LECTURE**, Fox Chase Cancer Center 13th Annual Postdoctoral Fellow and Graduate Student Symposium, Philadelphia, PA USA
- 2008 DOD BCRP Era of Hope Meeting 2008, Symposium Session: *Immune and Inflammatory Contributions to Breast Cancer*, AND *Era of Hope Spotlight Session*, Baltimore MD, USA
- 2008 *AACR Centennial Conference: Translational Cancer Medicine 2008: Cancer Clinical Trials and Personalized Medicine*; Hyatt Regency Monterey in Monterey, CA USA
- 2008 University of Michigan Comprehensive Cancer Center 2008 Fall Symposium, Ann Arbor MI, USA
- 2008 *AACR Special Conference, Chemical and Biological Aspects of Inflammation and Cancer*, Ko Olina Hawai, USA
- 2008 International Society for Biological Therapy of Cancer (iSBTc), Workshop on Inflammation in Cancer Development, Westin Horton Plaza San Diego, CA USA
- 2008 Skirball Symposium, New York University School of Medicine, New York, NY USA
- 2008 *AACR Special Conference in Cancer Research, Tumor Immunology: New Perspectives*; Miami FL, USA
- 2009 *1st Conference on Regulatory Myeloid Suppressor Cells*, Clearwater, FL USA
- 2009 Keystone Symposium, 'Extrinsic Control of Tumor Genesis', Vancouver, British Columbia CANADA
- 2009 *Inflammation and Cancer: Novel Aspects of Protumor Immunity*, Major Symposium, 100th Annual Meeting AACR, Denver CO USA
- 2009 2nd Annual Retreat of the CCR-NCI Cancer and Inflammation Program, Gettysburg, PA USA
- 2009 24th Annual Aspen Cancer Conference, Aspen, CO, USA
- 2009 2009 Geoffrey Beane Cancer Research Symposium: *Inflammation and Cancer*, Memorial-Sloane Kettering Cancer Center, New York NY USA
- 2009 AACR Special Conference, *Advances in Breast Cancer Research: Genetics, Biology, and Clinical Applications*, San Diego CA USA
- 2009 NCI's National Tumor Microenvironment Network, Nashville TN USA
- 2010 Joint Keystone Symposia, *Role of Inflammation in Oncogenesis/Molecular and Cellular Biology of Immune Escape in Cancer*, Keystone CO USA
- 2010 *3rd Annual Wyeth Discovery Frontiers in Human Disease Symposium*, New York, NY USA
- 2010 **PLENARY LECTURE**, 2010 Annual Meeting of the American Association for Cancer Research, Washington DC USA
- 2010 *10th Annual Oncology Research Symposium at MIT's Koch Institute for Integrative Cancer Research*. Boston MA USA
- 2010 *Metastasis and the Tumor Microenvironment*, Short Course, Eppley Institute for Cancer Research, Univ of Nebraska, Omaha, NB USA
- 2010 *Cancer Cell Biology and Signaling Workshop*, ImClone Systems/Eli Lilly, New York NY, USA

UPCOMING INVITATIONS

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- 2010 Symposium, Center for Excellence in Immunology of the National Cancer Institute, Bethesda MD, USA
- 2010 *25th Annual Critical Issues in Tumor Microenvironment, Angiogenesis and Metastasis*, Boston MA, USA
- 2010 Metastasis Research Society-AACR Joint Conference on *Metastasis and the Tumor Microenvironment*, Philadelphia, PA USA
- 2010 University of Vermont Cancer Center Symposia, *Inflammation and Cancer*, Burlington VT, USA
- 2010 **KEYNOTE LECTURE**, *Yves DeClerck Symposium*, Saban Research Institute, University of Southern California and Childrens Hospital Los Angeles, Los Angeles CA, USA
- 2010 **PLENARY LECTURE**, 2010 American College of Veterinary Pathologists and American Society for Veterinary Clinical Pathology, Concurrent Annual Meetings, Baltimore MD, USA
- 2011 2nd International Conference on Immunochemotherapy, entitled "*Immunochemotherapy: Correcting Immune Escape in Cancer*", Philadelphia PA USA
- 2011 Gordon Research Conference on *Epithelial Differentiation and Keratinization*, Snow Mountain, VT USA
- 2011 FASEB 2011, Steamboat Grand Resort Steamboat Springs, Colorado USA
- 2011 Gordon Research Conference on *Tissue Repair and Regeneration*, New Hampshire, USA

Invited Lectures/Seminars: International

- 2000 Medical Genome Center, Division of Molecular Medicine, Australian National University, Canberra, A.C.T. Australia.
- 2001 German Cancer Center, Heidelberg, Germany.
- 2001 MERCK Pharmaceutical, Darmstadt Germany.
- 2003 University of Toronto, Ontario Cancer Institute & Princess Margaret Hospital, Toronto, Ontario, CANADA
- 2004 Cancer Research UK, Barts & The London Queen Mary's School of Medicine & Dentistry, John Vane Science Center, Charterhouse Square, London, UK
- 2004 Cancer Research UK, London Research Institute, Lincoln's Inn Fields Laboratories, London, UK
- 2004 University of British Columbia, Department of Biochemistry and Molecular Biology, Vancouver, British Columbia, Canada
- 2007 Angiogenesis and Tumor Targeting Research Unit & Telethon Institute for Gene Therapy, San Raffaele Scientific Institute, Milan, ITALY
- 2008 Institute of Cell Biology, ETH Zurich Switzerland
- 2008 Institute of Cancer and the CR-UK Clinical Centre, Barts & The London School of Medicine and Dentistry, London UK
- 2009 University of South Hampton, UNITED KINGDOM
- 2009 The Netherlands Cancer Institute, Amsterdam, THE NETHERLANDS
- 2010 **DISTINGUISHED GUEST LECTURER**, Institute of Cancer, Barts & London School of Medicine. London GB

UPCOMING INVITATIONS

Invited Lectures/Seminars: National

- 1997 Biologic Therapy Research Conference. Univ. of Pittsburgh Medical Center, Pittsburgh, PA, USA
- 1997 Immunology Seminar Series. Univ. of Pittsburgh Medical Center, Pittsburgh, PA, USA
- 1999 Axys Pharmaceuticals, South San Francisco, CA, USA

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- 1999 Berlex Pharmaceuticals, Emeryville, CA, USA
- 1999 Axys Pharmaceuticals, La Jolla, CA, USA
- 1999 14th Annual Excalibur Round Table, American Cancer Society, San Francisco, CA, USA
- 1999 Colloquium in Microbiology, Cell and Molecular Biology. San Francisco State Univ., San Francisco, CA, USA
- 2000 Chiron Corporation, Emeryville, CA, USA
- 2000 Oral and Pharyngeal Cancer Branch/NIDCR, National Institutes of Health, Bethesda, MD, USA
- 2000 Fibrogen, Inc., South San Francisco, CA, USA
- 2000 Scios Inc., Sunnyvale, CA, USA
- 2000 Molecular Biology Department, University of Southern California, Los Angeles, CA, USA
- 2001 Dept. of Pediatric Hematology and Oncology, Children's Hospital Los Angeles, Univ. of Southern California, Los Angeles, CA, USA
- 2001 Jonnson Comprehensive Cancer Center, Univ. of Calif., Los Angeles, Los Angeles, CA, USA
- 2002 Institute for Engineering and Medicine, Univ. of Pennsylvania, Philadelphia, PA, USA
- 2002 Oncology Grand Rounds, Univ. of Missouri, Columbia, MO.
- 2002 Cancer Center, Univ. of California, Davis, Davis CA, USA
- 2002 AstraZeneca, Waltham, MA USA
- 2002 Pharmacology Seminar Series, Dept. of Pharmacology, Wayne State Univ., Detroit, MI, USA
- 2003 Dept. of Biology, Univ. of Calif., San Diego, San Diego, CA USA
- 2003 Tularik, Inc., South San Francisco, CA USA
- 2003 Dept. of Cancer Biology's Cancer Metastasis Research Program Seminar Series, M.D. Anderson Cancer Center, Univ. of Texas, Houston, TX, USA
- 2003 Dept. of Cancer Biology, Stanford University, Stanford, CA, USA
- 2004 Burnham Cancer Institute, San Diego, CA, USA
- 2004 The Wistar Cancer Institute, Philadelphia, PA, USA
- 2004 Regeneron Pharmaceuticals, Inc. Tarrytown, New York, USA
- 2004 Keynote Lecture: Vanderbilt University Digestive Disease Research Center Retreat, Vanderbilt University, Nashville, TN, USA
- 2004 Dana Farber Cancer Center, Harvard Medical School, Boston MA, USA
- 2004 Indiana University, Herman B. Wells Center for Pediatric Research and Clinical Cancer Center, Indianapolis IN, USA
- 2004 Immunology Graduate Program Seminar, Stanford University, Stanford, CA, USA
- 2005 Dept. of Nutritional Sciences & Toxicology, Univ. of Calif., Berkeley, Berkeley, CA USA
- 2005 Rigel, Inc., South San Francisco, CA USA
- 2005 Dept of Pathology & Lab Medicine, Univ. of California, Los Angeles, Los Angeles, CA USA
- 2006 Division of Cancer Biology and Angiogenesis in the Department of Pathology at Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA USA
- 2006 Department of Molecular and Medical Pharmacology, University of California, Los Angeles, Los Angeles, CA USA
- 2007 *Lymphoma and Myeloma Conference*, M.D. Anderson Cancer Center, Houston, TX, USA
- 2007 University of Minnesota, Dept. of Lab Medicine and Pathology, Minneapolis, MN, USA
- 2007 Memorial-Sloan Kettering Cancer Center, Program in Cancer Biology and Aging, New York NY, USA
- 2007 Abramson Family Cancer Research Institute and Univ. of Pennsylvania, Division of Hematology-Oncology, Philadelphia, PA USA
- 2007 Albert Einstein College of Medicine, New York NY, USA

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- 2007 Oncology Division Research, Biogen Idec Inc., San Diego, CA USA
- 2007 Genentech, Inc. Immunology Program. South San Francisco, CA USA
- 2007 University of Iowa Carver College of Medicine, Dept of Pathology, *Pathology Grand Rounds*, Iowa City, Iowa, USA
- 2007 Hollings Cancer Center, Medical University of South Carolina, Charleston, SC, USA
- 2007 University of Michigan, Program in Immunology and Cancer Research Series, Ann Arbor, MI USA
- 2008 Department of Pathology/UCLA School of Medicine Seminar, Los Angeles CA USA
- 2008 ANNUAL KEYNOTE LECTURE, Dept of Cancer Biology, Meharry Medical College, Nashville, TN USA
- 2008 University of California, Davis Cancer Center, Sacramento, CA USA
- 2008 Department of Immunology, University of Pittsburgh School of Medicine. Pittsburgh, PA, USA
- 2008 Cancer Biology Series, Ben May Cancer Center, University of Chicago, Chicago, IL, USA
- 2008 National Cancer Institute Center for Cancer Research Grand Rounds Series in Clinical and Molecular Oncology. Bethesda MD, USA
- 2009 University of Michigan, Oral Health Sciences Program and Biomedical Engineering Seminar Series, Ann Arbor, MI USA
- 2009 Department of Pharmacology, Wayne State University, Detroit, MI USA
- 2009 Molecular Biology Seminar Series, Biochemistry and Molecular Genetics, University of Colorado Health Sciences Center, Aurora, CO USA
- 2009 National Institutes of Health/National Cancer Institute, Vascular Biology Seminar Series, Bethesda MD, USA
- 2009 Genentech, Inc., Molecular Oncology Program. South San Francisco, CA USA
- 2009 Breast Cancer Network of Strength, California Breast Cancer Organizations, Northern California Affiliate, David CA USA
- 2009 Fred Hutchinson Cancer Center, Seattle WA USA
- 2010 Cold Spring Harbor Laboratory, CSH NY USA
- 2010 Albert Einstein College of Medicine, New York, NY USA
- 2010 Department of Cell Biology & Physiology Washington University, St Louis, MO USA
- 2010 Cancer Center Seminar Series at Burnham Institute for Medical Research, San Diego CA, USA
- 2010 Oncology Seminar Series, MedImmune, Gaithersburg, MD, USA

UPCOMING INVITATIONS

- 2010 Immunology Institute Seminar Series, Mt Sinai School of Medicine, NY, NY USA
- 2011 Hematology & Medical Oncology, Tulane Univ. School of Medicine, New Orleans, LA USA
- 2011 McArdle Seminar in Cancer Biology series, Univ of Wisconsin-Madison, USA
- 2011 Duke University Medical Center, Durham, North Carolina USA
- 2011 Tulane Cancer Center, Tulane University, New Orleans LA, USA
- 2011 Seminars in Oncology Lecture Series, Dana-Farber Cancer Institute and the Dana-Farber/Harvard Cancer Center, Boston MA, USA

Invited Lectures/Seminars: UCSF

- 1997 Breast Cancer SPORE Seminar. UCSF
- 1999 Cancer Research Institute Retreat, Tomales Bay, CA
- 2000 Chemistry and Cancer: How Chemistry-Based Tools Are Helping Solve Today's Serious Health Problems, Dev. & Alumni Relations, UCSF
- 2000 Oncology Grand Rounds, Department of Hematology and Oncology, UCSF

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- 2000 PIBS-Cell Biology Seminar Series, UCSF
- 2000 Pathology and Lab Medicine Grand Rounds, UCSF
- 2000 BMS Student Pizza Talk, UCSF
- 2000 Cell Cycle & Dysregulation Club, Comprehensive Cancer Center, UCSF
- 2000 Comprehensive Cancer Center Retreat, Granlibakken, Tahoe City, CA
- 2001 BMS Student Pizza Talk, UCSF
- 2001 Pathology and Lab Medicine Grand Rounds, Departments of Medicine and Pathology, UCSF
- 2001 UCSF, Cell Biology Retreat, Wilbur Hot Springs, CA, USA
- 2001 UCSF TETRAD Retreat, Granlibakken, Lake Tahoe, CA, USA
- 2001 UCSF Cancer Research Institute/BMS Retreat, Granlibakken, Lake Tahoe, CA. USA
- 2002 Current Topics in Medical Science, UCSF Medical Scientist Training Program (M170.09)
- 2002 Mouse Models of Human Cancer Program, Comprehensive Cancer Center, UCSF
- 2002 Cancer Research Institute Retreat, Santa Cruz, CA
- 2003 PIBS Student Pizza Talk, UCSF
- 2003 Breast Oncology Program, Comprehensive Cancer Center, UCSF
- 2003 Comprehensive Cancer Center Faculty Retreat: *Identification and Functional Assessment of Cancer Effectors*, Golden Gate Club, San Francisco CA
- 2004 BMS Graduate Program Retreat, Granlibakken Tahoe City, CA
- 2005 BMS Student Pizza Talk, UCSF
- 2006 *Introduction to Research*, Department of Pathology, UCSF
- 2008 Division of Experimental Medicine, Divisional Seminar Series, UCSF
- 2009 Immunology Program, UCSF
- 2009 Helen Diller Family Comprehensive Cancer Center *Research Symposium*; UCSF
- 2010 Bay Area Workshop on Lung Development, Physiology and Cancer, San Francisco CA USA
- 2010 UCSF-GIVI Center for AIDS Research (CFAR) Scientific Symposium for 2010: *HIV Infection, Inflammation, and Premature Aging*, San Francisco, CA USA
- 2010 Breast Oncology Program Seminar, Helen Diller Family Comprehensive Cancer Center, UCSF

UPCOMING INVITATIONS

- 2011 Helen Diller Family Comprehensive Cancer Center Symposium, San Francisco CA, USA

VII. GOVERNMENT AND OTHER PROFESSIONAL SERVICE:**GOVERNMENT SERVICE**

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|-----------|--|---|
| 2003-2006 | National Institutes of Health, Center for Scientific Review | Ad hoc reviewer (10/2003; 02/2005; 10/2005; 06/2006), Tumor Progression & Metastasis (TPM) Study Section, Oncological Sciences Review group |
| 2003 | Division of Cancer Biology, National Cancer Institute: <i>Microenvironment Think Tank</i> | Participant and <i>Reporter</i> |
| 2003 | Division Cancer Etiology, National Cancer Institute: <i>Validation of A Causal Relationship: Criteria to Establish Etiology Think Tank</i> | Invited speaker and Participant |
| 2004 | National Institutes of Health, National Cancer Institute | Subcommittee C (05/2004) – Basic & Preclinical NCI Initial Review Group, |

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2005	National Institutes of Health, National Cancer Institute	NCI-C RPRB (T2) Angiogenesis Subcommittee D (02/2005) – Clinical Studies NCI Initial Review Group, NCI-D RPRB Tumor Pathology
2005	National Institutes of Health, Center for Scientific Review-Oncology	Special Emphasis Panel (SEP); ZRG1 ONC (03) M, Developmental Therapeutics
2010	National Institutes of Health, Center for Scientific Review-Neuroscience	Special Emphasis Panel (SEP)/Scientific Review Group 2010/05 ZNS1 SRB-R (47)

OTHER PROFESSIONAL SERVICE

1999	Arkansas Science & Technology Authority	Ad hoc Grant Review
2000	McGraw-Hill, ' <i>Biology</i> ' 6 th edition, Ed. P.H. Raven and G.B. Johnson	Ad hoc Review, Chapters 17 and 18
2000	Division of Cancer Biology, NCI: <i>Epithelial-Stromal Interactions & Tumor Progression Workshop</i>	Invited speaker and Participant
2001	Department of Veterans Affairs	Ad hoc Grant Review, Oncology Review Board
2001	Research Grants Council of Hong Kong	Ad hoc Grant Review
2003	Danish Cancer Society, DENMARK	Ad hoc Grant Review
2004	Division of Gastroenterology and Digestive Disease Research Center, Vanderbilt University, Nashville TN, USA	' <i>H. pylori-induced Inflammation and Gastric Adenocarcinoma</i> ', PO1 External Advisory Panel
2004	Cancer Research Ireland, Irish Cancer Society	Ad hoc grant review
2004	Dutch Cancer Society	Ad hoc grant review
2004	Vanderbilt University, Nashville TN, USA; SPORE in GI Cancer	Ad hoc reviewer for SPORE Developmental Research Program
2005	Keystone Symposia, <i>Inflammation and Cancer</i>	Co-organizer (with Dr. Ray DuBois, Vanderbilt Univ, TN), Breckinridge, CO, USA
2006	5 th Annual Timberline Symp. on Epithelial Cell Biology, ' <i>Intrinsic and Microenvironmental Regulation of Epithelial Cancer</i> '	Co-Organizer (with Dr. Harold Moses, Vanderbilt University, TN, USA), Timberline, OR, USA
2006	Keystone Symposia Cancer Study Group for 2009 programming	Study group member
2007	Keystone Symposia, <i>Inflammation and Cancer</i>	Organizer (with Drs. Fran Balkwill (Cancer Research UK) and Glenn Dranoff (Beth Israel Cancer Center, Harvard, MA) Santa Fe, New Mexico, USA
2008	AACR Special Conference on ' <i>Inflammation and Cancer</i> '	Co-Organizer (with Drs. Michael Karin and Larry Marnett)
2007-2010	University of Minnesota Cancer Center; Douglas Yee, M.D., Director	External Scientific Advisory Board Member
2007 – 2011	University of Washington, Seattle WA, USA	Member, External Scientific Advisory Board, CA U54 TMEN: <i>Significance of Microenvironment for Prostate Cancer</i>

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2007 – 2011	Albert Einstein College of Medicine of Yeshiva University, New York, NY USA	<i>Initiation and Progression</i> ; P.I. Stephen R Plymate, Univ. of Washington School of Medicine. Member, External Scientific Advisory Board, CA U54 TMEN: <i>Novel Methods for Detection Cell Interactions in the Tumor Microenvironment</i> ; P.I. John S. Condeelis, Albert Einstein College of Medicine.
2008	International Society for Biological Therapy of Cancer (iSBTc), 2008 <i>Workshop on Inflammation in Cancer Development</i>	Co-Organizer (with Drs. Michael Karin, Steven Dubinett, George Weiner)
2009	GlaxoSmith Kline	Member, Tykerb Post-ASCO KOL Advisory Board
2009	University of Southern California, Children's Hospital	Member, External Scientific Advisory Board, Neuroblastoma, Program Project grant (P01), PI: Robert Seeger, M.D.,
2009-2011	Cancer Prevention and Research Institute of Texas (CPRIT)	Member, Scientific Review Committee; Basic Cancer Biology Review Committee

VIII. UNIVERSITY AND PUBLIC SERVICE

UNIVERSITY SERVICE

System wide

1992-1993	Graduate Student Representative, Dept. of Biological Chemistry Faculty Council, UCLA
2004	<i>ad hoc</i> Member External Advisory Panel; Jonsson Comprehensive Cancer Center, University of California, Los Angeles, Los Angeles CA, USA
2009	Member, Site Visit Programmatic Review Group, Department of Pathology & Laboratory Medicine, UCLA School of Medicine. Graduate Council of the UCLA Academic Senate.

University of California, San Francisco (CAMPUS-WIDE)

1997	Presentation, Donor Seminar, UCSF Development Office
1998	Presentation, Donor Seminar, UCSF Development Office
2000 – 2004	Member, Steering Committee, Ovarian Cancer Program Project Grant
2000 – 2005	Member, Scholarships and Awards Committee, Academic Senate, School of Medicine
2002 – present	Member, BioMedical Sciences Graduate Program (BMS) Executive Committee
2002 – 2004	Member, Medical Scientist Training Program Executive Committee
2004 - 2006	Member, Search Committee, Director of Molecular Imaging, Dept. of Radiology, Committee Chair: Ron Arenson, M.D. no successful recruitment
2004 – 2006	Member, BioMedical Sciences Graduate Program (BMS); Admissions Committee
2004	Organizer, BioMedical Sciences Graduate Program Retreat, Granlibakken, N. Lake Tahoe, CA USA
2005 - 2006	Member, Tissue Engineering Ladder-rank Faculty Search Committee, Dept. of Surgery. Committee Chair: Nancy Boudreau, Ph.D. Successful recruitment of Valerie Weaver, Ph.D.

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- 2005 - 2009 Member, Ethel and Jane Sokolow Memorial Cancer Endowment Lectureship Committee.
- 2006 Member, Cancer Faculty Search Committee, Anatomy Dept., Committee Chair: Zena Werb, Ph.D. Successful recruitment of Jeroen Roose, Ph.D.
- 2006 Member, Faculty Advisory Committee for 2007 Journalist Seminar on *Inflammation and Disease*. Sponsored by Associate Vice Chancellor Barbara J. French
- 2007 Member, committee to select recipient of Dean's Postdoctoral Prize Lecture.
- 2007 Member, Faculty Search Committee for Restorative Neurosurgery and Stem Cell Neurobiology, VA Medical Center/UCSF NeuroSurgery. Committee Chair: Linda Noble, Ph.D.; Status: open.
- 2009 Member, Committee to choose 1st Bonnie J. and Anthony Addario Endowed Chair in Thoracic Oncology, School of Medicine, UCSF
- 2010 Member, 2010 Selection Committee for the Hellman Family Early-Career Faculty Award.

University of California, San Francisco, Helen Diller Family Comprehensive Cancer Center

- 1999 Member, Cancer Center Research Building Space Review Policy Committee
- 1999 – 2002 Member, Mt Zion Animal Barrier Facility Committee
- 1999 – 2005 Member, Cancer Center Friday Seminar Series Committee
- 2000 Organizer and Chair, MZ Cancer Center Research Building Annual Retreat
- 2001 Member, 'Star Performance Award' selection committee
- 2001 Presentation, Evelyn Herman Reception, UCSF Development Office
- 2001 – 2002 Member, Cancer Center Research Building, 'Cancer Center Faculty Working Group'
- 2001 – 2006 Member, Mouse Models of Human Cancer Working Group
- 2002 – 2003 Member, UCSF Mt Zion campus, Animal Protocol Review Committee
- 2002 Member, ACS IRG grant review committee
- 2002 – 2006 Steering Committee Member, Mouse Models of Human Cancer
- 2003 Member, Review Committee, UCSF Comprehensive Cancer Center Stewart Trust Award
- 2003 – 2009 Chair, UCSF Mt Zion Campus Animal Protocol Review Committee
- 2003 Member, Search Committee: Associate Director for Administration, UCSF Comprehensive Cancer Center (Erica Weber, recruited)
- 2004 Member, Review Committee, UCSF Comprehensive Cancer Center Stewart Trust Award
- 2006 Co-Organizer, UCSF CCC Annual Symposium, *'Inflammation & Cancer: Bench to Bedside'*.
- 2008 Chair, Committee to nominate Postdoctoral scholar for AACR 2008 Annual Meeting, Inaugural "Future Leaders, New Directions" Special Symposium. Nominee: Laura Soucek, Ph.D. (awarded)

University of California, San Francisco, Cancer Research Institute

- 2001 – 2002 Member, Cancer Research Institute Membership Subcommittee

University of California, San Francisco, Department of Pathology

- 2003 Member, Committee to recommend faculty for the *Robert E. Smith Endowed Chair in Experimental Pathology*
- 2004 Member, Search Committee, Ladder rank faculty, Physician-Scientist, Anatomic Pathology. Successful recruitment of Jay Debnath, M.D., Ph.D.
- 2007 Member, Search Committee, Ladder-rank faculty, Physician-Scientist, Pathology

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and Neuropathology. Committee Chair: Michael D Prados, M.D.; Status: open.
 2008 Member, Search Committee, Ladder-rank faculty, Physician-Scientist, Experimental Pathology. Committee Chair: Benedict Yen, M.D.; Status: open
 2009-present Pathology Dept. Academic Merit and Promotions Committee

University (other)

2002 Guest Instructor, Graduate Oncology, University of Missouri, Columbia, Missouri USA
 2003 Guest Instructor, Cancer Biology, Stanford University, Stanford, CA USA
 2004 Guest Instructor, Immunology, Stanford University, Stanford, CA USA
 2008 Guest Instructor, *Exploring the Tumor microenvironment*, Postgraduate course, ISREC, Lausanne University's Biochemistry and Biology Departments, and the Lausanne Branch of the Ludwig Institute, Lausanne Switzerland. Course Organizers, Ivan Stamenkovic and Michel Aguet

PUBLIC SERVICE:

1990 Lecturer, Science Academy Of Whittier, Summer Institute. Whittier College, Whittier, CA
 1991 Organizer and Lecturer, Science Academy Of Whittier, Summer Institute. Whittier College, Whittier, CA.
 1993 Lecturer, Joslyn Community Center. Claremont, CA.
 1994 Provided elementary educators with science-related supplies (photos, slides, fixed tissue samples).
 1995 Co-Coordinator Hormone Research Institute, 'Take Our Daughters To Work Day', Univ. of Calif., San Francisco
 2002 Photo credits and interviewed for '*Misdiagnosis: Failure of Promising Cancer Treatment Starts Soul Searching by Researchers & Drug Companies*', in: *San Francisco Chronicle*, May 12, 2002.
 2003 Interviewed for article '*Body's First Defense May Be Root of Diseases*', in: *The Washington Post*, February 20, 2003
 2003 Interviewed for article '*The Body on Fire*', in: U.S. News & World Report, October 20, 2003
 2004 Interviewed for comments in: *Science News*, '*Early Warming: Inflammatory protein tied to colon cancer risk*' February 7, 2004, Vol 165.
 2004 Interviewed for article '*The Fires Within*', in: *TIME Magazine*, February 23, 2004
 2004 Interviewed for comments on AACR Annual Meeting in: *Oncology Times*, '*Exercise Reduces Inflammatory Response, May also Reduce Cancer Risk*', Robert H Carlson, 26(11):33-34, June10, 2004
 2004 Interviewed for article '*Inflammation and Cancer: The Link Grows Stronger*', in: *Science*, 306, 966-968 (2004)
 2005 Interviewed for article '*Quieting a Body's Defenses*', in: *Newsweek*, Special Edition, Summer 2005
 2006 Interviewed for "Expert Commentary" by *BreastLink.org*, on article "Association Between Circulating White Blood Cell Count and Cancer Mortality." *Archives of Internal Medicine*, January 23, 2006; 166:188-194. http://www.breastlink.org/index.php?module=announce&ANN_user_op=view&ANN_id=208
 2007 UCSF Research Perspectives 2007 – Inflammation as Cause and Consequences of Disease, Media Event for Journalists, September 27, 2007, UCSF Mission Bay Campus

2007 On-Air radio interview by Dave Iversen, KQED *FORUM*, September 28, 2007 San Francisco CA USA

IX. TEACHING AND MENTORING

Formal Scheduled Classes for UCSF Students:

Qtr	Academic Yr	Course No. & Title	Teaching Contribution	Units	Class Size
W	1997/98	IDS 100; Histology Laboratory	<i>Neoplastic Skin Histopathology</i> ; Laboratory lecture & instruction	10	150
W	1998/99	IDS 100; Histology Laboratory	<i>Neoplastic Skin Histopathology</i> ; Laboratory lecture & instruction	10	150
W	1999/00	IDS 100; Histology Laboratory	<i>Neoplastic Skin Histopathology</i> ; Laboratory lecture & instruction	10	150
S	1999/00	BMS 297A; Molecular Biology & Pathology of Neoplasia	<i>Animal Models of Cancer Laboratory</i> ; Laboratory lecture & instruction	3	15
S	2000/01	BMS 297A; Molecular Biology & Pathology of Neoplasia	<i>Animal Models of Cancer Laboratory</i> ; Laboratory lecture & instruction	3	15
W	2000/01	BMS 225; Tissue and Organ Biology	Lecture and laboratory instruction	3	15
S	2000/01	BMS 260; Cell Biology	Discussion group leader	1	6
F/W	2001/02	IDS 101; Prologue	Laboratory Instructor	9	30
W	2001/02	BMS 225; Tissue and Organ Biology	Lecture and laboratory instruction	3	15
W	2001/02	IDS 103; Cancer Block	<i>Invasion & Metastasis</i> ; Lecturer	7	150
S	2001/02	BMS 260; Cell Biology	Discussion group leader	1	7
F	2002/03	BMS 260; Cell Biology	Discussion group leader	1	6
W	2002/03	IDS 103; Cancer Block	<i>Invasion & Metastasis</i> ; Lecturer	7	150
F/W	2002/03	IDS 101; Prologue	Laboratory Instructor	9	30
F	2003/04	BMS 260; Cell Biology	Discussion group leader	1	6
S	2003/04	BMS 225B; Tissue and Organ Biology	Lecturer and Laboratory Instructor	1.5 - 5	tbd
W	2003/04	Biochem 297; Molecular Biology & Pathology of Neoplasia	<i>Angiogenesis</i> ; Lecturer	3	30
W	2003/04	BMS 297A Molecular Biology & Pathology of Neoplasia Laboratory	Lecturer and Laboratory Instructor, <i>Animal Models of Neoplasia</i>	1	10
S	2003/04	BMS 225B; Tissue & Organ Biology	Lecturer: Cancer I & Cancer II	1.5 - 5	16
F	2004/05	BMS 260; Cell Biology	Discussion group leader	1	6
F	2005/06	BMS 260; Cell Biology	Discussion group leader	1	7
W	2006/07	Biochem 297; Molecular Biology & Pathology of Neoplasia	<i>Inflammation and Cancer</i> ; Lecturer	3	30
W	2008/09	BMS230; Cellular & Molecular Biology of Cancer	Course Co-Director	3.5	22
W	2008/09	BMS230; Cellular & Molecular Biology of Cancer	Lecturer: <i>Cancer Microenvironments; Inflammation and Cancer</i>	3.5	22
W	2010/11	BMS230; Cellular & Molecular Biology of Cancer	Course Co-Director	3.5	22

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W	2010/11	BMS230; Cellular & Molecular Biology of Cancer	Lecturer: <i>Tumor cell heterogeneity; Cancer Microenvironments; Inflammation and Cancer</i>	3.5	22
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Postgraduate and Other Courses:

1989	M204, <i>Biochemistry Lab</i> Univ. of Calif., Los Angeles	Student Teaching Assistant for quarter long course (100 medical students)
1989	Biology 250, <i>Human Heredity</i> ; Dept. of Biology Whittier College, Whittier CA	Organized and taught entire lecture-based course (30 undergraduate students)
1990	Biology 350 & 350L, <i>Molecular Genetics</i> ; Dept. of Biology, Whittier College, Whittier CA	Organized and taught entire lecture and laboratory course (16 undergraduate students)
1990	M204, <i>Biochemistry Lab</i> Univ. of Calif., Los Angeles	Student Teaching Assistant for quarter long course (100 medical students)
1990	Biology 250, <i>Human Heredity</i> ; Dept. of Biology Whittier College, Whittier CA	Organized and taught entire lecture-based course (30 undergraduate students)
1992	Biology 350 & 350L, <i>Molecular Genetics</i> ; Dept. of Biology, Whittier College, Whittier CA	Organized and taught entire lecture and laboratory course (16 undergraduate students)
2003	Graduate <i>Oncology</i> , University of Missouri, Columbia, MS, USA	Invited Guest Lecturer: Lecture syllabus & delivered 2-hr lecture for course (15 students, combination of graduate, medical & postgraduate fellows)
2003	Graduate Program in Cancer Biology, Stanford Univ., Stanford, CA USA	Invited Guest Lecturer: Delivered 1-hr lecture to graduate students in Cancer Biology Graduate program
2004	Graduate Program in Immunology, Stanford Univ., Stanford, CA USA	Invited Guest Lecturer: Delivered 1-hr lecture to graduate students in Immunology Graduate program
2005	UCSF Dermatology residents' Basic Science Seminar Series	Invited Guest Lecturer: Delivered 1-hr lecture to UCSF Dermatology Residents (11 M.D. and M.D., Ph.D. Residents)
2008	ISREC, Lausanne University's Biochemistry and Biology Departments, and the Lausanne Branch of the Ludwig Institute	Guest Instructor: <i>Exploring the Tumor microenvironment</i> , postgraduate course. (20 PhD students, 3 hours of instruction)
2009	OOA Course: Tumor Microenvironment; The Netherlands Cancer Institute	Guest Faculty: (4.5 hours of instruction)
2010	25th Annual Harvard Tumor Course: Critical Issues In Tumor Microenvironment, Angiogenesis and Metastasis: <i>From Bench to Bedside and Back</i>	Faculty member: (2 hours of instruction)
2010	Eppley Institute for Research in Cancer, Univ. of Nebraska Medical Center. Short Course in Cancer Biology: Metastasis and the Tumor Microenvironment	Faculty member: (3 hours of instruction)

High School and Undergraduate Students Supervised or Mentored:

Dates	Name	Program or School	Faculty Role	Current position
1998	Christopher Tinkle	Undergraduate, Univ. of Texas, Austin, TX, USA	Summer Research Training Program Supervisor	MSTP student, Rockefeller University
2000	Adam Zucker	Undergraduate, Oberlin College, Ohio USA	Supervised Summer work	unknown

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2000	Ashkan Hirari	Undergraduate, Univ. of Calif., Berkeley, Berkeley CA, USA	Supervised Summer work	unknown
2001	Jason Reuter	Undergraduate, Univ. of Calif., Berkeley, Berkeley CA USA	Supervised Summer work	Ph.D. student, Stanford University
2002	Destinee Cooper	Undergraduate, Univ. of Calif., Davis USA	Summer Research Training Supervisor	unknown
2006	Sunum Mobin	UCSF Science & Health Education Partnership: High School Intern Program	Summer Research Training Supervisor	unknown
2008-2009	Julia Lam	Undergraduate, Univ. of Calif., Berkeley, Berkeley CA USA	Independent study (199), Mentor	B.S. U. C. Berkeley 2009
2010	Scott Keil	Undergraduate, The University of Glasgow, Scotland	Summer Research Training Supervisor	The University of Glasgow, Scotland
2010	Heather Chen	Undergraduate, Univ. of Calif., Berkeley, Berkeley CA USA	Summer Research Training Supervisor	Undergraduate, Univ. of Calif., Berkeley, Berkeley CA USA
2010	Amy Desalazar	Cupertino High School Cupertino, CA USA	Summer Research Training Supervisor	Cupertino High School
2010	Nikhil Wadhwani	Undergraduate, Sarah Lawrence College, Bronxville, NY USA	Summer Research Training Supervisor	Sarah Lawrence College, Bronxville, NY

Predocctoral Students Supervised or Mentored:

Dates	Name	Program or School	Faculty Role	Current position
2000	Jin-Sae Rhee	UCSF MSTP/BMS, graduate student	Rotation Supervisor	PhD awarded 2003, M.D. awarded 2005
2000 - 2003	Jin-Sae Rhee	UCSF M.D., Ph.D.,	Ph.D. supervisor	Pediatric Resident, Children's Hospital, Washington D.C.
2000	Maria Christophorou	UCSF BMS, graduate student	Faculty coach, BMS 297	Ph.D. awarded 2006
2001	Leslie Chu	UCSF BMS, graduate student	Rotation Supervisor	Ph.D. awarded 2005
2001	Rayna Takaki	UCSF BMS, graduate student	Rotation Supervisor	Ph.D. awarded 2006
2001 – 2002	Sophia Bruggerman	University of Nijmegen, The Netherlands	Masters Thesis Supervisor	Ph.D. student, The Netherlands Cancer Institute
2002	Lucy Lebedeva	UCSF PIBS, graduate student	Faculty coach, BMS 297	Ph.D. awarded 20057
2002	Leslie Chu	UCSF BMS, graduate student	Ph.D. Orals committee	Ph.D. awarded 2005
2002	Andre Whitkin	MSTP student, Cornell University USA	Supervised Summer work	MSTP student, Cornell University
2002	Karin deVisser	The Netherlands Cancer Institute, The Netherlands	Ph.D. Thesis Reading Committee	Postdoctoral fellow, The Netherlands Cancer Institute
2003	Cathy Collins	UCSF MSTP student	MSTP Advisor	MSTP student, UCSF
2004	Eric Tamm	University of British Columbia, Canada	Doctoral Dissertation External Examiner	Postdoctoral fellow, Genentech Inc.,
2004	Annie Hsieh	University of Södertörn, Sweden	Masters Thesis Supervisor	unknown

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2005	Geoff Benton	UCSF TETRAD/PIBS, graduate student	Ph.D. Orals committee	UCSF TETRAD PhD graduate student
2006	Morgan Truitt	UCSF BMS, graduate student	Rotation Supervisor	UCSF BMS PhD graduate student
2006	Danielle Shin	UCSF MSTP student	Rotation Supervisor	MSTP student, UCSF
2006-2008	Celeste Rivera	SFSU/UCSF NIH Post-baccalaureate Research Experience Program (PREP) student	M.S. research advisor	current MS student, Coussens lab UCSF
2007-present	Leslie Vasquez	SFSU/UCSF NIH Post-baccalaureate Research Experience Program (PREP) student	M.S. research advisor	current MS student, Coussens lab UCSF
2008	Ashley Martin	UCSF BMS, graduate student	Rotation Supervisor	UCSF BMS PhD graduate student
2009	Kay Wiebrands	Master's Student Utrecht University, the Netherlands	Masters Thesis Internship Supervisor	Master's Student Utrecht University, the Netherlands
2009-2010	David Tawfik	Medical Student III, UCSF	MSIII break year. Dean's Quarterly Research Fellowship; PACCTR Fall Quarter Fellowship;	MSIII, UCSF
2009-present	Renee Vanderlaan	UCSF BMS, graduate student	Chair: Thesis Committee	UCSF BMS graduate student, Lab of Matthias Hebrook, Ph.D.
2009-present	A. Preethi Ganessan, M.D.	Ph.D. Graduate Student (Cancer Research UK, Univ of Southampton)	Ph.D. supervisor	Current Ph.D. student, Coussens lab
2010-present	Lucia Cottone	Ph.D. graduate student (San Raffaele Institute, Milan Italy)	Ph.D. supervisor	Current Ph.D. student, Coussens lab

Postdoctoral Fellows and Residents Directly Supervised or Mentored

Dates	Name	Position & Funding	Faculty Role	Current Position
2000 - 2001	Ernst Lengyel, M.D., Ph.D.	Post-Doc Researcher, Senior Clinical Fellow	Research Supervisor	Assoc. Adj. Prof., Dept. Gyn. & Oncology, UCSF
2000 -2002	Leon Van Kempen, Ph.D.	Post-Doc Researcher, Dutch Cancer Society Postdoctoral Fellowship	Research Supervisor	Asst Prof., Univ. of Nijmegen, Dept. of Pathology, The Netherlands
2002 – 2005	Robert Diaz, Ph.D.	Post-Doc Researcher; Coussens R01	Research Supervisor	Scientist, Roche Pharmaceuticals
2002 – 2005	Karin deVisser, Ph.D.	Post-Doc Researcher, Dutch Cancer Society Postdoctoral Fellowship	Research Supervisor	Research Scientist, The Netherlands Cancer Institute, Amsterdam, The Netherlands
2003 – 2007	Alexandra Eichten, Ph.D.	Post-Doc Researcher, Serono Fndt for the Advancement of Medical Science (2003-2005);	Research Supervisor	Scientist, Regeneron Corp., New York USA
2003 - 2005	Stephen Robinson, Ph.D.	Post-Doc Researcher; Coussens R01	Research Supervisor	Private sector, United Kingdom
2003 - 2004	H. Jennifer Shen, Ph.D.	Post-Doc Researcher; Coussens R01	Research Supervisor	Post-Doctoral fellow, NIH
2005 -	David DeNardo, Ph.D.	Post-Doc Researcher;	Research	Post-Doctoral fellow,

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present		1) NGA: 5 T32 CA09043 PI: BISHOP; <i>Molecular Analysis of Tumor Viruses</i> ; 2) American Cancer Society Postdoctoral Fellowship 2007-2010	Supervisor	Coussens Lab, UCSF
2005 –2007	Nor Eddine Sounni, Ph.D.	Post-Doc Researcher; Coussens R01	Research Supervisor	Research Scientist, Univ. of Liege, Belgium
2006 –2007	Tingting Tan, M.D.,Ph.D.	Post-Doc Researcher; Coussens R01	Research Supervisor	Resident, Internal Medicine, Kaiser San Francisco
2006 - present	Magnus Johansson, Ph.D.	Post-Doc Researcher; Sweedish Cancer Society Postdoctoral fellowship 2006-2008	Research Supervisor	Post-Doctoral fellow, Coussens Lab, UCSF
2006-present	Nessrine Affara, Ph.D.	Post-Doc Researcher; AACR-Astellas USA Fndt in Basic Cancer Research 2009-2010	Research Supervisor	Post-Doctoral fellow, Coussens Lab, UCSF
2007 -2010	Pauline Andreu, Ph.D.	Post-Doc Researcher; Cancer Research Institute Postdoctoral Fellowship 2008-2011	Research Supervisor	Research Funding Agency in Private Sector, France
2008-present	Brian Ruffell, Ph.D.	Post-Doc Researcher; Dept of Defense Postdoctoral fellowship 2009-2012	Research Supervisor	Post-Doctoral fellow, Coussens Lab, UCSF
2009-present	Stephen Shiao, M.D., Ph.D.	UCSF Radiation Oncology Resident	Research Supervisor	UCSF Radiation Oncology Fellowship

FACULTY MENTORING

Faculty Mentored:

Dates	Name	Position while Mentored	Mentoring Role	Current Position
2001 – 2004	Ernst Lengyel, M.D., Ph.D.	Asst. Adjunct Professor	Research Mentor	Asst. Prof., Dept. Gyn. & Oncology, Univ. of Chicago, Chicago, IL
2002 – 2007	Darya Soto, M.D.	Asst. Adjunct Professor,	K08 Research Mentor	Private Practice, Burlingame, CA
2005 – 2007	Runi Chattopadhyay, M.D.	Clinical Instructor and Clinical Fellow	Basic Science Mentor, K12	Director, Breast Center, California Pacific Med. Center, San Francisco CA
2006 – 2009	Limin Liu, Ph.D.	Assistant Professor	Member, Mentoring Committee	Dept. of Microbiology & Immunology, Sandler Center for Basic Research in Asthma, UCSF
2010-present	Jaynata Debnath, M.D., Ph.D.,	Assistant Professor	Faculty Mentor	Dept. of Pathology, UCSF

Sabbatical Visitors:

1999 - 2000 Yves DeClerck, M.D. Professor, Univ. of Southern Calif. & Children's Hospital of Los Angeles

SUMMARY OF TEACHING HOURS

Academic Year	Teaching/Mentoring Summary	Hours
1997/98	<u>Total hours of teaching /mentoring:</u> Formal class or course teaching hours: Informal teaching hours including prep time: Mentoring hours:	<u>27</u> 2 1 24
1998/99	<u>Total hours of teaching /mentoring:</u> Formal class or course teaching hours: Informal teaching hours including prep time: Mentoring hours:	<u>71</u> 2 1 68
1999/00	<u>Total hours of teaching /mentoring:</u> Formal class or course teaching hours: Informal teaching hours including prep time: Mentoring hours:	<u>108</u> 4 2 102
2000/01	<u>Total hours of teaching /mentoring:</u> Formal class or course teaching hours: Informal teaching hours including prep time: Mentoring hours:	<u>130</u> 16 9 105
2001/02	<u>Total hours of teaching /mentoring:</u> Formal class or course teaching hours: Informal teaching hours including prep time: Mentoring hours:	<u>201</u> 18 19 164
2002/03	<u>Total hours of teaching /mentoring:</u> Formal class or course teaching hours: Informal teaching hours including prep time: Mentoring hours:	<u>314.5</u> 15.5 17 282
2003/04	<u>Total hours of teaching /mentoring:</u> Formal class or course teaching hours: Informal teaching hours including prep time: Mentoring hours:	<u>402</u> 20 28 354
2004/05	<u>Total hours of teaching /mentoring:</u> Formal class or course teaching hours: Informal teaching hours including prep time: Mentoring hours:	<u>395</u> 17 28 350
2005/06	<u>Total hours of teaching /mentoring:</u> Formal class or course teaching hours: Informal teaching hours including prep	<u>395</u> 17 28

	time: 350 Mentoring hours:	
2006/2007	<u>Total hours of teaching /mentoring:</u> 473 Formal class or course teaching hours: 45 Informal teaching hours including prep time: 28 Mentoring hours: 400	
2008/2009	<u>Total hours of teaching /mentoring:</u> 499 Formal class or course teaching hours: 51 Informal teaching hours including prep time: 48 Mentoring hours: 400	
2009/2010	<u>Total hours of teaching /mentoring:</u> 499 Formal class or course teaching hours: 51 Informal teaching hours including prep time: 48 Mentoring hours: 400	
2010/2011	<u>Total hours of teaching /mentoring:</u> 499 Formal class or course teaching hours: 51 Informal teaching hours including prep time: 48 Mentoring hours: 400	

X. RESEARCH AND CREATIVE ACTIVITIES

RESEARCH AWARDS AND GRANTS:

CURRENT

R01CA140943 (multi-PI: Coussens, Boudreau, Daldrup-Link; UCSF) 07/01/09 –06/30/13

Source: NIH/NCI

Title: Improved Imaging and Drug Delivery Using Novel Approaches to Regulate Tissue Perfusion

The major goal of this project is to examine how short-term inhibition of ALK5 in vivo alters hemodynamics and tissue perfusion in mouse models of cancer.

Role: P.I.

RO1 CA130980 (PI: Coussens, LM; UCSF)

07/01/08-06/30/13

Source: NIH/NCI

Title: Regulation of Inflammation-Associated Epithelial Cancer Development

Role: Principal Investigator

The goal of this study is to determine regulatory programs activating chronic inflammation during squamous carcinogenesis

RO1 CA132566 (multi PI: Coussens, LM; Jablons DM; UCSF)

05/01/08-04/30/13

Source: NIH/NCI

Title: Inflammation and Lung Carcinogenesis

Role: Principal Investigator

The goal of this study is to determine how inflammation and Wnt signaling regulate stem cell

 niche autonomy during lung carcinogenesis

W81XWH-08-PRMRP-IIRA (multi-PI: Broaddus, C; Coussens, LM; UCSF) 07/01/09 –06/30/12

Source: DOD

Title: Role of Macrophage-induced Inflammation in Mesothelioma

The goals of this project are 1) to determine the functional significance of macrophage phenotype in mesothelioma, 2) to determine the functional significance of macrophages as regulators of mesothelioma apoptosis in vitro and 3) to define the functional significance of macrophage depletion or repolarization on mesothelioma survival in vivo.

Role: P.I.

P50 CA58207 (Gray; LBNL/UCSF)

08/01/92–11/30/12

Source: NIH/NCI

Title: Bay Area Breast Cancer SPORE

Career Development and Developmental Research Award, Multi Project PI: Boudreau N; Coussens LM

Title: Macrophage-Mediated Delivery of the Breast Tumor Suppressor HoxD10 via Autologous Transfer to Breast Tumors. The aims of this project are to 1) establish function and optimize introduction of the engineered HoxD10 protein into macrophages and/or monocytes; 2) visualization of modified monocyte/macrophage accumulation in mammary tumors in vivo and 3) analysis of the impact of monocyte/macrophage delivered HoxD10 on breast tumor growth, progression and metastasis in MMTV-PyMT mouse model of mammary carcinogenesis.

Role: Multi P.I.

P30 CA82103 (PI: McCormick, F; UCSF)

08/5/99-05/31/12

Source: NIH/NCI

Title: *Cancer Center Support Grant*

Role: Co-Director, Mouse Pathology Shared Resource

The Cancer Center Support Grant provides support for administration and infrastructure for the UCSF Comprehensive Cancer Center. Dr. Coussens is the Core Co-Director of the Mouse Pathology Shared Resource that provides routine hematologic and histopathologic processing of tissue and blood samples to members of the UCSF community.

BC051640 Era of Hope Scholar Award (PI: Coussens, LM; UCSF)

06/01/06 – 05/31/11

Source: Department of Defense Breast Cancer Research Program (BCRP) of the Office of the Congressionally Directed Medical Research Programs (CDMRP)

Title: Microenvironment Regulation of Mammary Carcinogenesis

Role: PI

The goal of this Scholar Award is to identify leukocytes and their proteases that modify breast carcinogenesis and to develop noninvasive imaging reagents targeting leukocytes to image inflammation.

PREVIOUS

USPHS 5 T32 CA09056 (PI: Fox, F, UCLA)

07/01/89 –06/30/92

Source: NIH/UCLA

Title: Regulation of *junB* Gene Expression by TGF-Beta

Competitive Pre-Doctoral award to study transcription factor *junB*.

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Univ. of Calif., Dissertation Year Fellowship (PI: Coussens, LM, UCLA) 10/1/92 – 09/31/93
Source: University of California, Office of the President
Title: *Effects of E1A on TGF-Beta-inducible junB Expression*
 Competitive Pre-Doctoral award to study transcription factor *junB*.

USPHS 5 T32 CA09043 (PI: Bishop, KM, UCSF) 10/01/93-06/31/96
Source: NIH/UCSF
Title: *Molecular Analysis of Tumor Viruses*
 Post-Doctoral fellowship to study mouse model of epithelial carcinogenesis.

American Social Health Association/Pfizer Post-Doctoral Research Fellowship in Sexually Transmitted Diseases (PI: Coussens, LM, UCSF) 10/01/96 – 9/30/98
Source: Private Foundation
Title: *Metalloproteinases and Malignant Progression of Squamous Epithelium in K14-HPV16 Transgenic Mice*
Role: Principal Investigator
 Competitive Post-Doctoral fellowship to study proteases and tumor development

P01 CA072006 (PI: Shuman M, UCSF) 06/10/97 – 06/30/03
Source: NIH/NCI
Title: *Proteases in Cancer Biology and Drug Development*
 Project 3 – Proteases in Models of Tumor Initiation/Progression
Role: Co-Investigator, Project 3
 The major goal of this project is to study the role of proteases in cancer biology.

Core C – Transgenic Animal Models
Role: Director (year 4 and 5)
 The major goal of this Core is to develop and provide protease null and transgenic mice to program projects.

UCSF IRG-97-150-01 (PI: Coussens LM, UCSF) 07/01/99-06/30/00
Source: American Cancer Society
Title: *Proteases and Genomics in a Mouse Model of Epithelial Cancer*
Role: Principal Investigator
 Pilot project tested role of proteinases as effectors of genomic instability.

UCSF Cell Cycle and Dysregulation Program (PI: Coussens LM, UCSF) 02/01/00-01/31/01
Source: UCSF Comprehensive Cancer Center, Intramural
Title: *Epithelial Neoplastic Progression and Degradation of Type I Collagen*

Lisa M. Coussens, Ph.D.

Role: Principal Investigator

Pilot project assessed functional significance of type I collagen metabolism during epithelial carcinogenesis.

Research Evaluation and Allocation Committee (PI: Coussens LM, UCSF) 07/01/00-06/30/01

Source: UCSF Academic Senate**Title:** *Role of Gelatinase B in Maintenance of Genomic Instability***Role:** Principal Investigator

Pilot project tested the role of MMP9 as an indirect regulator of genomic instability.

UCSF IRG AC-04-02 (PI: Coussens LM, UCSF) 10/1/00-09/30/01

Source: American Cancer Society**Title:** *Regulation of Intracellular Signaling Pathways by Gelatinase B/MMP-9***Role:** Principal Investigator

Pilot project to study signal transduction pathways regulated by MMP-9.

The V Foundation for Cancer Research (PI: Coussens LM, UCSF) 06/02/00-05/31/02

Source: Private Foundation**Title:** *Gelatinase B and Epithelial Cancer Development***Role:** Principal Investigator

Pilot project to study role of MMP9 during epithelial carcinogenesis.

Gertrude B. Elion Cancer Research Award (PI: Coussens LM, UCSF) 07/1/01 – 06/30/02

Source: American Association of Cancer Research**Title:** *Functional Role of MMP-2 During Epithelial Carcinogenesis***Role:** Principal Investigator

Pilot project to study role of MMP-2 during epithelial carcinogenesis.

Univ. of Calif., Cancer Research Coordinating Committee (PI: Coussens LM, UCSF) 07/01/01 – 06/30/02

Source: University of California**Title:** *Gelatinase A/MMP-2 and Epithelial Cancer Development***Role:** Principal Investigator

Pilot project to study role of MMP-2 as a potentiator of tumor development.

Hellman Family Award For Early Career Faculty (PI: Coussens LM, UCSF) 11/01/00-09/30/02

Source: UCSF Intramural**Title:** *Paracrine Regulation of Epithelial Carcinogenesis by MMP-9***Role:** Principal Investigator

Pilot project to identify matrix molecules regulated by MMP-9.

Edward Mallinckrodt, Jr. Foundation (PI: Coussens LM, UCSF) 10/01/00-09/30/03

Source: Private Foundation**Title:** *Regulation of epithelial cancer by gelatinase B/MMP-9*

Lisa M. Coussens, Ph.D.

Role: Principal Investigator

Pilot project to determine how MMP-9 regulates proliferation, VEGF bioavailability and angiogenesis during epithelial carcinogenesis.

P50 CA58207 (PI: Gray, J: UCSF)

03/01/03-02/28/05

Source: NIH/NCI

Bay Area Breast Cancer Translational Research Program (SPORE)

Title: *Type I Collagen Remodeling and Mammary Carcinogenesis***Role:** Principal Investigator (Developmental Project)

The overall goal of this pilot project was to explore the role of collagen metabolism during mammary carcinogenesis.

DE-FG02-05ER6401 (PI: Franc, B; UCSF)

03/01/05 – 01/16/06

Source: DOE Medical Applications Grant

Title: Therapeutic Radionuclide Tumor-targeting Strategy for Breast Cancer

directs

Role: Co-Investigator

The specific aim of this project is to develop a radionuclide delivery molecule (RDM) that specifically targets cancer cells that express matrix-metalloproteinase-14 (MMP-14) on their surface and demonstrate delivery of radiolabeled RDM to MMP-14 expressing cells *in vitro* and *in vivo*.

R01 DK067678 (PI: Cher, M: Wayne State University)

07/01/03-06/30/06

Source: NIH/NIDDK**Title:** *Proteases in Prostate Cancer Bone Metastasis***Role:** Subcontract Principal Investigator

The major goal of this subcontract is to assist with the planned experiments by providing mice (protease deficient) of defined genotype for proposed studies to analyze proteases during prostate metastasis to bone *in vivo*.

Opportunity Award, Sandler Family (PI: Coussens, LM; UCSF)

02/15/05 -02/14/07

Source: UCSF Intramural**Title:** *B Lymphocytes as Targets for Cancer Prevention***Role:** Principal Investigator

The major goal of this project is to investigate the efficacy of targeting B cells for chemoprevention

DAMD17-02-1-0693 (PI: Sloane, B; Wayne State University)

08/01/02-07/31/06

Source: Department of Defense**Breast Cancer Center of Excellence**

Title: *Validation of Proteases as Therapeutic Targets in Breast Cancer Functional Imaging of Protease Expression, Activity and Inhibition*

Role: Subcontract Principal Investigator

The goal of this program is to validate proteases as therapeutic targets in breast cancer by functional imaging of protease expression, activity and inhibition.

R01 CA94168 (PI: Coussens, LM: UCSF)

04/01/02-06/31/07

Source: NIH/NCI**Title:** *Regulation of Epithelial Cancer by MMP-9/gelatinase B*

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Role: Principal Investigator

The goal of this project is to identify molecules that mediate proliferative and cellular pathways activated by MMP-9.

U54 RR020843 (PI: Smith, J; Burnham Institute)

09/30/04-07/31/06

Source: NIH/National Center for Research Resources**Title:** Center on Proteolytic Pathways**Role:** Principal Investigator (Driving Biological Problem #1)DBP#1 *Proteolytic Pathways in Acute Vascular Response***P01 CA72006** (PI: Werb, Z; UCSF)

07/07/03 – 06/30/08

Source: NIH/NCI**Title:** *Proteases in Cancer Biology and Drug Development -*Project 3 - *Proteases in Models of Tumor Initiation/Progression***Role:** Co-Investigator, Project 3 -

The major goal of this project is to study the role of proteases in cancer biology.

Core C - *Transgenic Animal Models***Role:** Director

The major goal of this Core is to develop and provide protease null and transgenic mice to program projects.

R01 CA98075 (PI: Coussens, LM; UCSF)

07/01/03-06/30/09

Source: NIH/NCI**Title:** *Microenvironmental Regulation of Tumor Progression***Role:** Principal Investigator

The overall goal of this grant is to determine the role of collagen metabolism on epithelial carcinogenesis.

XI. PEER REVIEWED PUBLICATIONS

1. Francke U, de Martinville B, **Coussens L**, Ullrich A. (1983) The human gene for the Beta subunit of nerve growth factor is located on the proximal short arm of chromosome 1. *Science* 222:1248-1251.
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recognition particle receptor in the endoplasmic reticulum membrane. *Nature* 318:334-338.

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9. Parker P, **Coussens L**, Totty N, Rhee L, Young S, Chen E, Stabel S, Waterfield M, Ullrich A. (1986) The complete primary structure of protein kinase C—the major phorbol ester receptor. *Science* 233:853-859.
10. **Coussens L**, Parker P, Rhee L, Yang-Feng T, Chen E, Waterfield M, Francke U, Ullrich A. (1986) Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. *Science* 233:859-866.
11. **Coussens L**, Rhee L, Parker P, Ullrich A. (1987) Alternative splicing increases the diversity of the human protein kinase C family. *DNA* 6:389-394.
12. Yarden Y, Kuang W-J, Yang-Feng T, **Coussens L**, Munemitsu S, Dull T, Schlessinger J, Francke U, Ullrich A. (1987) Human proto-oncogene *c-kit*: A new cell surface receptor-tyrosine kinase for an unidentified ligand. *EMBO J.* 6:3341-3351.
13. MacDonald R, Pfeffer S, **Coussens L**, Tepper M, Brocklebank C, Mole J, Anderson J, Chen E, Czech M, Ullrich A. (1988) A single receptor binds both insulin-like growth factor II and mannose-6-phosphate. *Science* 239:1134-1137.
14. Formby B, Ullrich A, **Coussens L**, Walker L, Peterson C. (1988) Growth hormone stimulates insulin gene expression in cultured human fetal pancreatic islets. *J. Clin. Endo. Metab.* 66:1075-1079.
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32. de Visser KE, Korets LV, **Coussens LM**. (2004) Early neoplastic progression is complement-independent. *Neoplasia* 6: 768-776.
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 - *Cancer Cell* (2005), 7: 403-405. 'The role of the immune system in early carcinogenesis: B-ware the double edged sword'. A. Houghton, H. Uchi, and J.D. Wolchok.
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44. DeNardo D **Coussens LM**. (2007) Balancing Immune Response: Crosstalk Between Adaptive and Innate Immune Cells During Breast Cancer Progression. *Breast Cancer Res*, 9:212-222. [PMCID: PMC2206719](#)
45. Kopitz C, Gerg M, Bandapalli O, Ister D, Pennington CJ, Hauser S, Flechsig C, Krell HW, Antolovic D, Brew K, Nagase H, Stangl M, Hann von Weyhern CW, Brucher BLDM, Brand K, **Coussens LM**, Edwards DR, Kruger A. (2007) TIMP-1 promotes liver metastasis by induction of HGF-signaling. *Cancer Research*, 67:8615-8623. [PMID: 17875701](#)
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 - *Nature Biotechnology* (2009) 27:828. 'Promoting metastasis' by L. DeFrancesco
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Manuscripts Submitted or In Revision:

- Egeblad M, Wiseman BS, Sternlicht MD, Green KA, Kouros-Mehr H, DeNardo D, Wilcox J, Bissel MJ, **Coussens LM**, Lund LR, Werb Z. Matrix metalloproteinase-dependent remodeling of the collagen scaffold regulates mammary epithelial invasion. *Manuscript submitted*
- Okamoto J, Mikami I, Raz DJ, Segal M, Yagui-Beltran A, Johansson A, **Coussens LM**, Chen Z, Zhou HM, Hirata T, Clement G, Koizumi K, Shimizu K, Jablons DM, He B. Down regulation of EMX2 is associated with clinical outcome in lung adenocarcinoma, *Manuscript submitted*

XII. PATENTS

1. U.S. Patent Application Serial No. 10/567,873
Title: *Novel Indications for Transforming Growth Factor-Beta Regulators*.
Inventors: **Lisa M. Coussens** and Zena Werb
Application published on August 28, 2008 as U.S. Patent Publication #2008-0206219-A1
International filing date: August 9, 2004. Application Filing Number: 60/493,643; Docket Number: 23540-09361/PCT; International Publication Number WO 2005/013915 A2; International Application Number: PCT/US2004/025902
2. DeNardo D, Brennan D, **Coussens LM**. *Phenotyping Tumor-Infiltrating Leukocytes*, Provisional patent application number: 61/227,035

XIII. NON-PEER REVIEWED PUBLICATIONS AND EDITORIALS:**Review Articles, Symposium Proceedings and Editorials**

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Book Chapters

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XIV. RESEARCH PROGRAM:

The Coussens lab focuses on the role of immune cells and leukocyte proteases as critical

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regulators of skin, lung and breast cancer development. During the early development of cancer, many physiological processes occur in the vicinity of 'young tumor cells' that are similar to processes that occur during embryonic development and to healing of wounds in adult tissue, e.g., inflammation, angiogenesis (development of new blood supply) and tissue remodeling. During tumor development; however, instead of initiating a 'healing' response, activated leukocytes provide growth-promoting factors that help tumors grow. We are interested in understanding the molecular mechanisms that regulate leukocyte recruitment into neoplastic tissue and subsequent regulation those leukocytes exert on evolving cancer cells. To address these issues, we have taken several approaches to investigate mechanisms involved in: *i.* induction and maintenance of chronic inflammatory microenvironments in premalignant, malignant and metastatic tissues, *ii.* role of leukocyte proteases as regulators of tissue remodeling, angiogenesis and cancer development, and *iii.* development of novel non-invasive imaging reagents to monitor immune response in tissues/tumors. The long-term goal of this work is to translate basic observations made in the mouse, toward rational design of novel therapeutics whose aim will be to block and/or alter rate-limiting events critical for solid tumor growth or maintenance in humans. Currently, we are actively utilizing transgenic mouse models of cancer development, including non-small cell lung cancer, skin and breast cancer, and mesothelioma to reveal the functional roles of adaptive and innate leukocytes during solid tumor development. These experimental studies are conducted in parallel with evaluation of representative human cancer specimens to affirm that mechanisms revealed in the experimental setting represent fundamental parameters of multi-stage cancer development in humans.



XV. MOST SIGNIFICANT PUBLICATIONS

1. de Visser KE, Korets LV, **Coussens LM**. (2004) Early neoplastic progression is complement-independent. *Neoplasia* 6: 768-776.

Role: This study was based on the hypothesis that inflammation accompanying premalignant progression in HPV16 mice was dependent upon activation and mobilization of the Complement cascade. To address this, my laboratory generated Complement protein 3 (C3)-deficient HPV16 mice and assessed the functional significance of C3 by its absence during neoplastic progression. These studies revealed that abundant deposition of C3 is a characteristic feature of premalignant hyperplasias and dysplasias coincident with leukocyte infiltration in neoplastic tissue, genetic elimination of C3 neither affects inflammatory cell recruitment towards neoplastic skin, nor impacts responding pathways downstream of inflammatory cell activation, e.g. keratinocyte hyperproliferation or angiogenesis. Taken together, these data suggest that complement-independent pathways are critical for leukocyte recruitment into neoplastic tissue and leukocyte-mediated potentiation of tumorigenesis. 100% of the research supporting this manuscript was conceived and conducted in my laboratory. de Visser wrote the manuscript under Dr. Coussens' direct supervision.

2. de Visser KE, Korets LV, **Coussens LM**. (2005) De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer Cell*, 7:411-423.

Role: Chronic inflammation predisposes tissue to cancer development; however, regulatory mechanisms underlying recruitment of innate leukocytes toward developing neoplasms are obscure. In this manuscript, we reported that genetic elimination of mature T and B lymphocytes in HPV16 mice, limits neoplastic progression to development of epithelial hyperplasias that fail to recruit innate immune cells. Adoptive transfer of B lymphocytes or serum from HPV16 mice into T and B cell-deficient/HPV16 mice restored innate immune cell infiltration into premalignant tissue and reinstated necessary parameters for full malignancy, e.g., chronic inflammation, angiogenic vasculature, hyperproliferative epidermis. These findings support a model in which acquired immunity is required for establishing chronic inflammatory states that promote de novo carcinogenesis. This report received a great deal of attention as it represented the first such report linking adaptive immune responses during the earliest stages of epithelial carcinogenesis - a paradigm shift from current thinking that revealed provocative new targets for anti-cancer therapy. This manuscript was the "featured article" in its issue of *Cancer Cell*, and was the subject of several invited review articles (Houghton et al., *Cancer Cell* 2005; Montavani, *Nature* 2005), as well as being featured in 'Research Highlights' in *Nature Reviews Cancer* and *Nature Reviews Immunology* (Minton, 2005). 100% of the research supporting this manuscript was conceived and conducted in my laboratory. de Visser wrote drafts of the manuscript under Dr. Coussens' direct supervision.

3. DeNardo DG, Baretto JB, Andreu P, Vasquez L, Kolhatkar N, **Coussens LM**. (2009) CD4⁺ T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. *Cancer Cell*, 16:91-102.

Role: Infiltration of T lymphocytes in human breast cancers has been recognized by pathologist for decades, however their functional role has been undetermined. We utilized a transgenic mouse model of mammary carcinogenesis and demonstrated a tumor-promoting role for T_H2-CD4⁺ T lymphocytes that elicit pro-tumor, as opposed to cytotoxic bioactivities of tumor-associated macrophages and enhancement of pro-metastatic epidermal growth factor receptor signaling programs in malignant mammary epithelial cells. This work revealed a novel pro-tumor regulatory program involving components of the acquired and cellular immune systems that

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effectively collaborate to promote pulmonary metastasis of mammary adenocarcinomas, and identified new cellular targets, namely CD4⁺ T effector cells and IL-4 for anti-cancer therapy. This manuscript appeared as the “featured article” in the August 4th 2009 issue of *Cancer Cell*, and was the subject of an invited “Preview” article (Pardoll, *Cancer Cell* 2009), and was featured in the research highlights section of *Nature Reviews Cancer* (McCarthy, 2009). 100% of the research supporting this manuscript was conceived and conducted in my laboratory. Dr. DeNardo wrote drafts of the manuscript under Dr. Coussens’ direct supervision.

4. Andreu P, Johansson M, Affara NI, Tan TT, Junankar S, Korets L, Lam J, Tawfik D, Pucci F, De Palma M, DeNardo D, de Visser KE, **Coussens LM**. (2010) FcR γ activation regulates inflammation-associated squamous carcinogenesis. *Cancer Cell*, 17(2):121-134.

Role: This work presents novel findings demonstrating the functional significance of Fc γ receptors and humoral immunity as potentiators of squamous carcinogenesis. While myeloid cells and some T cell subsets have been implicated in neoplastic progression and cancer development, the tumor-promoting capabilities of B lymphocytes have remained unclear. Using the HPV16 transgenic mouse model of inflammation-associated squamous carcinogenesis, we previously reported that B and T cell-deficient HPV16 mice failed to progress beyond a benign hyperplastic state due to deficient activation of chronic inflammatory programs in early neoplastic skin (deVisser et al, *Cancer Cell* 2005). In Andreu et al., we revealed that B cells potentiate squamous carcinogenesis via humoral immunity, where immunoglobulins (Ig) in the form of immune complexes (IC) activate Fc γ receptor-mediated signaling pathways on resident and recruited myeloid cells. Activation of these programs on resident mast cells initially leads to peripheral blood leukocyte recruitment into neoplastic skin and activation of angiogenic vasculature. The subsequent chronic inflammation that ensues in part maintains angiogenic support but also supports neoplastic keratinocyte hyperproliferation and progression to dysplastic/carcinoma *in situ* states and subsequent malignant conversion and carcinoma development. These novel findings have clinical significance in that they imply that anti-cancer strategies targeting B cells, Ig or FcR γ may harbor therapeutic efficacy in limiting risk of malignant conversion in patients suffering from chronic inflammatory diseases, or in patients harboring premalignant lesions whose molecular and/or immunologic characteristics favor tumor development. This manuscript appeared as the “featured article” in the March 2010 issue of *Cancer Cell*, and was the subject of an invited “Preview” article (Mantovani, *Cancer Cell* 2010), and was featured in the research highlights section of *Nature Reviews Immunology* (Byrd, 2010). 100% of the research supporting this manuscript was conceived and conducted in my laboratory. Dra. Andreu, Johansson and Affara wrote drafts of the manuscript under Dr. Coussens’ direct supervision.

5. Sounni NE, Dehne K, vanKempen LCL, Egeblad M, Affara NI, Cuevas I, Wiesen J, Junankar S, Korets L, Lee J, Shen J, Morrison C, Overall CM, Krane SM, Werb Z, Boudreau N, **Coussens LM**. (2010) Stromal regulation of vessel stability by MMP14 and TGF β . *Disease Model. Mech.* 3:317-332.

Role: In patients with locally advanced solid tumors, first-line treatment is often neo-adjuvant or pre-operative chemotherapy, which helps shrink tumors before surgery, allowing for more conservative surgical approaches and reducing the potential for developing systemic disease. However, despite aggressive chemotherapy, long-term survival for many patients remains poor, in part owing to limitations with the targeting and accumulation of cytotoxic drugs in tumor tissue. The vasculature of solid tumors is abnormal, both in terms of vessel architecture and the dynamics of blood flow. Permeable heterogeneous vessel walls allow the leakage of proteins and fluid that, coupled with the inefficiency of lymphatic drainage, could be exploited to develop novel,

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enhanced drug delivery strategies that are therapeutically selective and improve clinical outcome. This work describes a previously unappreciated role for transforming growth factor beta (TGF β) in regulating vascular stability and vessel permeability in solid tumors. Using mouse models, we demonstrated an endogenous pathway that regulates normal vascular permeability, which is controlled by perivascular collagen, the metalloproteinase enzyme MMP14, and TGF β . In wild-type mice, inhibitors of either MMP14 or TGF β signaling induce blood vessel permeability. Conversely, enhanced MMP14 or TGF β activity in the mouse epidermis decreases leakage across cutaneous vessels. This pathway remains functional during tumor progression, as acute blockade of either MMP14 or TGF β signaling transiently alters vessel stability, 'opening' vascular beds and promoting intravenous delivery of high molecular weight compounds to the tumor. This implying that delivery of standard therapeutic agents or diagnostic molecular imaging agents to tumor tissue may be enhanced by transient blockade of the TGF β pathway. If so, this could advance disease therapy and/or diagnostic imaging, not only in cancer medicine, but also in fibrotic disorders such as scleroderma and kidney failure. Research in support of this manuscript spanned over 7 years and many post doctoral fellows and technicians. The initial hypothesis and ideas towards successful completion of the project were conceived by Dr. Coussens, who also wrote the manuscript with input from Drs. Boudreau and Werb.

Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity

David G. DeNardo · Pauline Andreu · Lisa M. Coussens

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Abstract Tumor-associated myeloid cells have been implicated in regulating many of the “hallmarks of cancer” and thus fostering solid tumor development and metastasis. However, the same innate leukocytes also participate in anti-tumor immunity and restraint of malignant disease. While many factors regulate the propensity of myeloid cells to promote or repress cancerous growths, polarized adaptive immune responses by B and T lymphocytes have been identified as regulators of many aspects of myeloid cell biology by specifically regulating their functional capabilities. Here, we detail the diversity of heterogeneous B and T lymphocyte populations and their impacts on solid tumor development through their abilities to regulate myeloid cell function in solid tumors.

Keywords Cancer · Inflammation · Lymphocyte · Macrophage · Metastasis

1 Introduction

Virchow first described leukocyte infiltration of solid tumors in the 1800s; however, only recently have we begun to understand the diverse regulatory roles played by immune cells during cancer development. Historically,

leukocytes found in and around developing tumors were thought to represent an attempt by the host to eradicate neoplastic cells. Indeed, some leukocytes, including CD8⁺ cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, do play a critical role in restraining tumor development [1]. However, we now appreciate that the significance of these anti-tumor programs can be thwarted by other subsets of leukocytes that instead foster tumor development [2–5]. Immune-competent mouse models of human cancer have enabled a detailed evaluation of the tumor-promoting capacity of several subsets of myeloid cells, including mast cells (MCs), monocytes, granulocytes/neutrophils, and macrophages, as well as some subsets of lymphocytes [6, 7]. However, depending on their differentiation status and immune microenvironment, subpopulations of these same cells can also support tumor rejection and response to anti-cancer therapy [2, 8, 9], thus indicating that pro- and anti-tumor programming of leukocytes is dynamic. In this review, we discuss recent insights into the role of B and T lymphocytes as “gatekeepers” of myeloid cell bioactivity (Fig. 1) and how recognition of these dynamic interactions reveals novel opportunities for anti-cancer therapy.

2 Paradoxical role of CD4⁺ T lymphocytes in solid tumor development

In contrast to CD8⁺ CTLs that play well-defined roles in hindering cancer development, the functional significance of CD4⁺ T lymphocytes in tumor progression appears more paradoxical. For example, retrospective evaluation of colon and lung carcinomas revealed that extensive infiltration of tumors by CD4⁺ T cells correlates with favorable clinical outcome, whereas in breast and renal cancers exhibiting similar infiltrations instead correlates with decreased overall

D. G. DeNardo · P. Andreu · L. M. Coussens (✉)
Department of Pathology, University of California,
513 Parnassus Ave., HSW-450C,
San Francisco, CA 94143, USA
e-mail: Lisa.Coussens@ucsf.edu

L. M. Coussens
Helen Diller Family Comprehensive Cancer Center,
University of California,
513 Parnassus Ave., HSW-450C,
San Francisco, CA 94143, USA

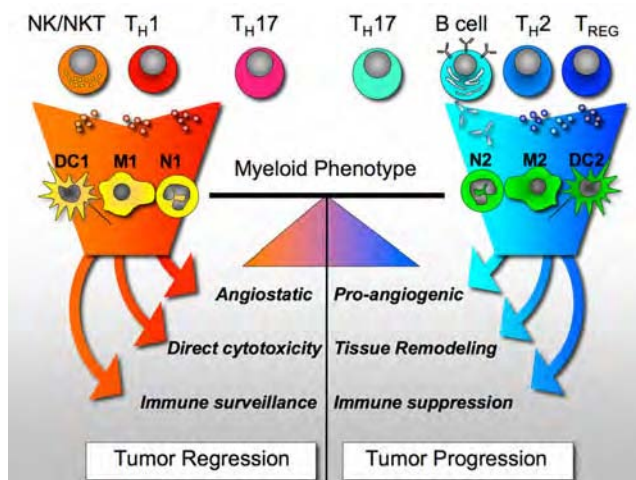


Fig. 1 Adaptive immune responses control tumor-associated myeloid cell bioactivity and tumor progression. Polarized responses by adaptive immune cells alter the balance between pro- and anti-tumor myeloid cell bioactivities. When the host's response to neoplastic cell growth results in the production of T_H1 cytokines by $CD4^+$ T lymphocytes and NK cells, myeloid cells in turn induce programs promoting tumor regression and/or dormant disease. However, when these adaptive immune responses include chronic B lymphocyte activation and IgG production in combination with T_H2 and T_{REG} lymphocyte activation, programs of immune suppression, angiogenesis, tissue remodeling, and invasion are favored in myeloid cells and contribute to tumor progression and metastasis

survival [10–13]. Analysis of mouse models of human cancer have provided some clarity for these disparate findings and revealed that etiology and organ specificity matters with regards to how $CD4^+$ T cells aid or constrain tumor progression. Schreiber and colleagues demonstrated that, whereas $CD4^+$ T cell deficiency enhances methylcholanthrene (MCA)-initiated sarcoma development [14], carcinoma development on the other hand is inhibited following two-stage squamous carcinogenesis [15, 16]. Similarly, in a mouse model of skin and cervical carcinoma development where oncogenes from human papilloma virus type 16 (HPV16) are expressed under the control of the keratin 14 promoter, skin carcinoma formation is modestly attenuated by $CD4^+$ T cell deficiency, whereas cervical carcinoma development is significantly enhanced [17, 18]. Together, these observations demonstrate that immune responses accompanying tumor development are organ dependent and, based on the neoplastic microenvironment, engage either pro- or anti-tumor immune programs. The heterogeneity of $CD4^+$ T cell subsets that accumulate in tissues may be at the heart of these paradoxical findings.

3 $CD4^+$ T lymphocyte heterogeneity

$CD4^+$ T cells represent a highly heterogeneous population of cells that develop along different functional lineages

depending upon polarizing cytokine signals during activation by antigens [19]. Classically, $CD4^+$ T lymphocyte subsets include T_H1 and T_H2 lineages that are characteristically fostered by exposure to interleukin (IL)12 and IL4, respectively. Following activation, $CD4^+$ T cells assuming the T_H1 fate secrete interferon (IFN) γ , tumor necrosis factor (TNF) α , IL2, and IL12 [20]. Through the production of these cytokines, T_H1 cells regulate immune surveillance programs by up-regulating antigen processing and presentation on major histocompatibility complex (MHC) I and II molecules by professional antigen presenting cells (APCs) and as such can regulate the duration and magnitude of $CD8^+$ CTL responses [21]. In addition, following strong antigen-specific activation, T_H1 cells can directly kill tumor cells by releasing high levels of IFN γ , TNF α and cytolytic granules. Thus, when present, T_H1 responses can directly and indirectly regulate anti-tumor programs that restrain cancer development.

In contrast, T_H2 $CD4^+$ T cells express high levels of IL4, -5, -6, -10, and -13 that, together, alter adaptive immunity by inducing T cell anergy, inhibiting T cell-mediated cytotoxicity as well as fostering humoral immune responses directed by B cells [22, 23]. The T_H2 cytokines IL4 and IL13 are important mediators of $CD4^+$ T cell functionality *in vitro*, T_H2 $CD4^+$ T cells inhibit apoptosis and induce proliferation of breast carcinoma cells; *in vivo*, IL4 emanating from $CD4^+$ T cells fosters breast cancer growth [24, 25]. Consistent with these findings, a high ratio of T_H2^+ to T_H1^+ cells correlates with parameters of clinical disease progression, such as increased tumor size and grade and lymph node metastasis of breast cancers [26].

Adding to this T_H1 versus T_H2 paradigm, $CD4$ lineages have recently expanded to include a T_H17 subset that is differentiated by a combination of IL6 and transforming growth factor (TGF) β and mediate their effects through secretion of IL17, -21, and -22, [27–29]. T_H17 cells are thought to play an important role in protection against some extracellular pathogens and in regulating auto-immune disease [30, 31]. As such, T_H17 cells have been implicated in the development of inflammation-associated colonic tumors in response to pathogenic bacteria [32]. T_H17 cell infiltration has also been observed in patients with colon, ovarian, prostate, and hepatocellular carcinoma where high numbers of IL17-producing cells correlates with poor prognosis [33–35]. In mouse models of non-small cell lung cancer (NSCLC), IL17 enhanced tumor growth by promoting development of angiogenic vasculature [36, 37]. In contrast, in a B16 melanoma model, IL17 depletion rendered mice more susceptible to metastasis [38], a phenotype that was blocked by adoptive transfer of tumor-specific T_H17 cells that fostered immune surveillance by $CD8^+$ CTLs and dendritic cells (DCs) [38]. Together, these experimental findings indicate that the role

of T_H17 cells in regulating aspects of cancer development may also be context dependent.

In addition to T_H1 , T_H2 , and T_H17 $CD4^+$ effectors, populations of $CD4^+FoxP3^+$ T regulatory (T_{REG}) cells are also thought to play a considerable role in regulating tumor immunity. In human cancers, increased prevalence of $CD4^+FoxP3^+$ T_{REG} cells correlates with increased survival for follicular lymphoma [39], while it instead correlates with poor prognosis in pancreatic ductal carcinoma [40], non-small cell lung cancer [41], renal cell carcinoma [11], and breast carcinoma [42]. Suppression of the anti-tumor activities of $CD8^+$ CTLs, NKs, and DCs is at the heart of how T_{REG} cells control tumor development [43, 44]; however, the multitude of mechanisms whereby they regulate anti-tumor programs suggest the existence of distinct tissue-specific subpopulations of T_{REG} cells, each endowed with or capable of various bioeffector activities [45–47]. Mechanistic studies have revealed that T_{REG} cells support pro-tumor immunity not only by increasing local levels of immunosuppressive cytokines including $TGF\beta$, IL35, and IL10 but also by direct cytolytic effects through production of perforin and granzyme. In addition, T_{REG} cells can disrupt the metabolic activity of cyclic adenosine monophosphate (cAMP) transfer, as well as inhibit APC function by inducing binding of CTLA-4 to CD80/86 [44, 48].

Thus, while it is now clear that a spectrum of $CD4^+$ T cell subtypes are present in human tumors of essentially all types, the role they play in promoting or repressing tumor development likely has to do with the type of $CD4^+$ T cell subtype that is either recruited to or accumulates within each distinct tumor microenvironment. These in turn then regulate anti-tumor programs by professional cytotoxic cells ($CD8^+$ T CTLs and NK cells), as well as regulating pro-tumor properties of a diverse array of myeloid cell subtypes as discussed below.

4 Myeloid heterogeneity and tumor development

Innate immune cells of myeloid origin, e.g., granulocytes (neutrophils, basophils, and eosinophils), DCs, macrophages, NK cells, and MCs, are also prominent components of pre- and malignant tissues and functionally contribute to cancer development by releasing a myriad of cytokines, chemokines, matrix metalloproteinases, serine proteases, DNA-damaging molecules (reactive oxygen species), histamine, and other bioactive mediators that regulate tissue remodelling and angiogenesis [49–53], suppress anti-tumor immunity [54–56], and enhance tumor cell survival, migration, and metastasis [57, 58].

Nucleated hematopoietic cells that have been directly implicated in tumor angiogenesis include MCs [51], tumor-associated macrophages (TAMs) [5, 23, 59], Tie2-

expressing monocytes [50, 60], neutrophils [52], DC precursors [61], and myeloid immune suppressor cells [62, 63]. Other hematopoietic cell types, such as platelets [64], eosinophils [65], and hematopoietic progenitors [66], also participate in angiogenic processes, but it remains to be established whether they can directly promote tumor angiogenesis, rather than having a broader function in supporting tissue inflammation and remodelling.

In contrast, these same myeloid cell lineages also foster tumor rejection by inducing angiostatic programs, enhancing CTL and NK responses, and directly inducing tumor cell death [13]. As an example of these paradoxical roles, studies from several laboratories have reported that TAMs enhance angiogenesis and metastasis of malignant mammary tumors [25, 67, 68]. In contrast, TAMs exposed to toll-like receptor (TLR) ligands and/or $IFN\gamma$ directly lyse mammary tumor cells, increase antigen presentation, and secrete angiostatic proteins such as CXCL10 and 11 [8, 9, 69]. These distinct bioactivities are mirrored in neutrophils, MCs, and DCs and may be due to the inherent plasticity of myeloid lineage cells regulated by local factors present in distinct tissue and/or organ microenvironments.

The bioactive states of macrophages, as well as other myeloid cells, have been classified according to T_H1 and T_H2 nomenclature, referred to as M1 (classical) or M2 (alternative) activation, respectively [2, 70, 71] (Fig. 1). M1 macrophages are regulated by T_H1 cytokines including $IFN\gamma$, $TNF\alpha$, and granulocyte monocyte-colony stimulating factor (GM-CSF) that enhance macrophage cytotoxic activity, production of pro-inflammatory cytokines, and antigen presentation capacity [70, 71]. In contrast, tissue macrophages can achieve various alternatively activated M2 states following exposure to T_H2 cytokines, including IL4 or IL13 (M2a), potentiation by immune complexes and TLR ligands (M2b) or immunosuppressive cytokines including IL10 or $TGF\beta$ and/or glucocorticoid hormones (M2c) [70].

The general hallmarks of M2 macrophages include high levels of IL10, IL1Ra, IL1 decoy receptor CCL17 and CCL22 secretion, high expression of mannose, scavenger and galactose-type receptors, low expression of IL12, as well as poor APC capability. Intriguingly, although these alternative activation states (M2a, b, and c) share many phenotypic characteristics, they are distinct and induce individual context-dependent environmental responses. For example, induction of an M2c phenotype by IL10 results in highly immune suppressive macrophages that can also produce matrix components such as versican or PTX3. In contrast, T_H2 cytokine induction of M2a TAMs induces expression of fibronectin, as well as catabolism of L-arginine by arginase that in turn leads to increased collagen synthesis and matrix remodeling [70, 72]. Our own work has revealed that IL4 and/or IL13 activation of macro-

phages induces production of growth factors including epidermal growth factor, TGF β , and basic fibroblast growth factor that together regulate invasive, angiogenic, and immune-suppressive programs [25] (unpublished data). Both M2a and M2b macrophages down-regulate the pro-inflammatory cytokines IL1, IL6, and TNF α [73], whereas induction of M2b macrophages by immune complexes induces these same inflammatory cytokines in addition to IL10 and also likely enhances vascular responses such as endothelial migration and vessel dilation [74]. Thus, M2-polarized cells promote scavenging of debris, angiogenesis, and remodeling and repair of wounded/damaged tissues. Parallel and non-redundant activity states have also been defined for DCs (i.e., DC1, DC2) [75] and neutrophils (i.e., N1, N2) [76] (Fig. 1).

To address pro- versus anti-tumor capabilities of these opposing states, Hagemann and colleagues demonstrated that by “reprogramming” M2 TAMs through deletion of IKK β macrophage phenotype could be switched from immunosuppressive to actively promoting immune surveillance, as reflected by decreases in IL10 and arginase-1 expression and increased IL12 and nitric oxide production together resulting in decreased ovarian tumor growth through recruitment and activation of NK cells [67, 77]. Again, organ specificity and/or etiology may play a role in regulating how reprogramming can be achieved. In a mouse model of squamous carcinogenesis, we recently reported that Fc γ R signaling in myeloid cells directly regulates whether myeloid cells enhance or repress cancer development, which correlate with their unique gene expression signatures that reflect M1 versus M2 and DC1 versus DC2 programs [74]. These data indicate the significance of reprogramming myeloid cell phenotypes to affect tumor outcome. The major question that arises with regards to this capability then becomes what are the cellular and molecular programs in tissues and/or tumors that regulate the bioactive state of these important myeloid cells and how recognition of these can be translated into anti-cancer therapy.

5 T lymphocytes as regulators of anti-tumor macrophages

Establishment of an immune reaction in homeostatic tissue typically involves activation of NK cells in response to stress signals or infectious agents, whom by their production of IFN γ in turn prime macrophages towards an M1 state, culminating in enhanced presence of macrophages with cytotoxic capability [71]. However, production of IFN γ by NK cells is generally transient and therefore insufficient to sustain M1 macrophage polarization; thus, IFN γ -producing T_H1 cells are critical for immune responses requiring sustained M1 macrophage bioactivities. In tumors, studies

by Corthay and colleagues demonstrated that T_H1 cell regulation of locally activated M1 macrophages were significant and fostered rejection of myeloma and lymphomas in the absence of CTL responses [69, 78]. Moreover, expression of IL12 and IFN γ by T_H1 cells can combine to enhance anti-tumor responses by NK and NK-T cells by up-regulating expression of NK receptors such as NKG2D (in response to IL12) and expression of NK receptor ligands such as RAE1 on target cells (in response to IFN γ) [79]. T_H1 responses then in turn favor anti-tumor NK and macrophage responses that eliminate neoplastic cells. However, while T_H1 cells are antigen specific, tumoricidal macrophages exert indiscriminate cell killing activity. Thus, multiple immunosuppressive programs have evolved to eliminate the adverse autoimmune pathologies, such as rheumatoid arthritis, that are associated with over-activation of these M1 responses [80]; unfortunately, many of these immunosuppressive programs are usurped by developing cancers.

6 T lymphocytes as regulators of pro-tumor myeloid cells

In contrast to induction of tumor-immune surveillance programs by T_H1 cells, T_{REG} and T_H2 cells have the capacity to induce alternative activation states of macrophages, DCs, and neutrophils involved in promoting cancer development. Studies of human T_{REG}s have demonstrated their ability to block classical activation of macrophages and instead foster immunosuppressive myeloid phenotypes through the production of IL10 and TGF β [81]. Similar biology may apply to neutrophils, as recently reported by Fridlender and colleagues who found that loss of TGF β signaling through ALK4/5 inhibition resulted in recruitment of N1-polarized neutrophils with tumoricidal bioactivities [76]. While TGF β in the tumor microenvironment is produced by multiple cell types, these data may indicate that T_{REG} cells suppress N1 tumoricidal responses through production of TGF β and, as such, favor pro-tumor N2 or immature monocyte (IMC) phenotypes. Our own work has demonstrated that CD4⁺ T_H2 cells in mammary tumors promote M2 responses in TAM and IMCs that in turn enhance pulmonary metastasis [25]. Together, these data indicate that the balance of T_H1 versus T_H2/T_{REG} responses regulates the pro- versus anti-tumor programming of tumor-associated myeloid cells.

7 B lymphocytes as regulators of myeloid cells during cancer development

B lymphocytes constitute a central component of humoral immunity and not only serve in antibody production but also in antigen presentation and cytokine secretion. In

particular, B lymphocyte expression of MHC and co-stimulatory molecules as well as secretion of pro-inflammatory cytokines are critical for regulating CD4⁺ and CD8⁺ T cell activation, expansion, antigenic spreading, and memory T lymphocyte formation. The heterogeneity of B lymphocyte responses has been recently recognized and diverse B cell subtypes with either pro-immune or regulatory properties have been identified *in vivo*. Precisely, regulatory B lymphocytes (B_{REG}), which include various subtypes of IL10-producing cells, have been identified in the context of autoimmune diseases and exert anti-inflammatory functions [82, 83]. However, a role for B_{REG} cells in cancer has not been fully elucidated.

B lymphocytes in general have only recently gained recognition for representing significant components of tumor immunity [84]. B cell involvement in solid tumor development was initially described in syngeneic allograft murine tumor models in combination with genetic or antibody-mediated B cell depletion. In these studies, B cell-deficient mice (μ MT) exhibited resistance to several types of syngeneic tumors, including EL4 thymoma, MC38 colon carcinoma, and B16 and D5 melanoma [85, 86], whereas partial B cell depletion resulted in significantly reduced tumor burden in a transplantable model of colorectal cancer [87]. A tumor-promoting role for B cells in solid tumor development was also revealed in transgenic mice expressing tumor necrosis factor (TNF)-receptor-associated factor 3 (TRAF3) in lymphocytes [88]. TRAF3⁺ lymphocytes induce humoral immune responses leading to chronic inflammation and a significantly elevated incidence of squamous cell carcinomas [88]. These experimental findings indicate that, in the absence of an initiating oncogenic event, B lymphocyte-mediated chronic inflammation is sufficient to foster solid tumor formation. In contrast, an opposite and anti-tumor immune surveillance role for B lymphocytes has also been demonstrated in a syngeneic melanoma model where deletion of mature B cells by anti-CD20 IgG significantly enhanced tumor growth and metastasis [80], suggesting that the role of B lymphocytes in tumor progression, like CD4⁺ T lymphocytes, may be context dependent and driven by individual B lymphocyte subtype specificity.

Mechanistically, B cells and humoral immunity can act to modulate solid tumor development by regulation of diverse effector pathways involving secretion of pro-inflammatory, as opposed to regulatory, cytokines, e.g., IL10, TGF β , inhibition of CTL activity [89], perturbation of T_H1/T_H2 CD4⁺ T cell lineages [90, 91], as well as differential recruitment and activation of innate immune cells [89, 92].

Recently, using a transgenic mouse model of inflammation-associated carcinogenesis, i.e., K14-HPV16 mice [93], we revealed a novel pathway by which B lymphocytes enhance squamous carcinogenesis and demonstrated the significance of the B cell/immunoglobulin/Fc γ R signaling axis. We found

that B cells and humoral immunity fostered cancer development by activating Fc γ receptors on resident and recruited myeloid cells [74]. Stromal accumulation of autoantibodies in premalignant skin, through their interaction with activating Fc γ Rs, regulated recruitment, composition, and bioeffector functions of leukocytes, in particular subsets of tumor-promoting polarized myeloid cells in neoplastic tissue which in turn enhanced neoplastic progression and subsequent carcinoma development [74]. A similar pro-tumor role for B cells was recently reported by Ammirante and colleagues who found that B cells are critical for growth of castration-resistant prostate cancer metastasis, not through production of immunoglobulins or regulation of Fc γ R signaling but instead by delivery of lymphotoxin that in turn activates IKK- α and STAT3 in prostate cancer cells and subsequently stimulates metastasis by an NF- κ B-independent, cell-autonomous mechanism [94]. These findings together with other experimental studies support a model in which B cells, through various mechanisms, including humoral immunity, activating Fc γ Rs and IKK, are required for establishing chronic inflammatory programs that promote *de novo* carcinogenesis.

8 Conclusions

While many factors regulate the propensity of immune cells to promote or repress solid tumor development, polarized adaptive immune responses by B and T lymphocytes can specifically regulate multiple pro-tumor properties of myeloid cells that in turn control many of the “hallmarks” of cancer development [95, 96]. Thus, recognition of the soluble molecules that mediate these important paracrine interactions may represent critical targets to evaluate for anti-cancer therapy. Importantly, targeting of pro-tumor pathways that neutralize M2-type macrophage and/or T_H2-type CD4⁺ T cell responses and therein foster M1 or T_H1-type immunity may enhance sensitivity to cytotoxic therapies, including chemo- and radiation therapy, whose durability may be limited by the longevity of the anti-tumor immune responses that they induce.

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LISA M. COUSSENS, PH.D.

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REGULATION OF PROTUMOR IMMUNITY AND CANCER DEVELOPMENT**LISA M. COUSSENS, PH.D.**

Department of Pathology and Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA 94143, USA. Email: Lisa.Coussens@ucsf.edu

Clinical and experimental studies have established that chronic infiltration of neoplastic tissue by leukocytes, i.e., chronic inflammation, promotes development and/or progression of various solid tumors^{1,2}; however, the organ-specific cellular and molecular programs that favor pro-tumor, as opposed to anti-tumor immunity by leukocytes are incompletely understood. While some leukocytes certainly exhibit anti-tumor activity, i.e., cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells³, other leukocytes, most notably mast cells, CD4⁺ T cells, B lymphocytes, dendritic cells (DCs), granulocytes, immature monocytes and macrophages exhibit more bipolar roles, by virtue of their capacity to either hinder or potentiate tumor progression^{1,2}. A major question regarding these disparate leukocytes bioactivities is the degree to which their various pro- or anti-cancer activities are regulated by tissue-specificity and/or are responsive to individual oncogenic or tumor suppressive gene programming in early neoplastic tissue.

Leukocytes and Breast Carcinogenesis: In the breast, cancer development is in part characterized by a significant increase in both innate and adaptive immune cells, with B and T lymphocytes and macrophages representing the most abundant leukocytes present in neoplastic stroma⁴. Retrospective clinical studies examining identity of leukocytes in human breast cancer have revealed that high immunoglobulin (Ig) levels in tumor stroma (and serum), and increased presence of extra follicular B cells, T regulatory (T_{reg}) cells, high ratios of CD4/CD8 or T_H2/T_H1 T lymphocytes in primary tumors or in draining lymph nodes correlates with tumor grade, stage and overall patient survival³. On the other hand, experimental studies have demonstrated that macrophages in primary mammary adenocarcinomas regulate late-stage carcinogenesis by virtue of their pro-angiogenic properties^{5,6}, as well as fostering pulmonary metastasis by providing epidermal growth factor (EGF) and cathepsin proteases⁷ to malignant mammary epithelial cells (MECs) and thereby enhancing their invasive (and metastatic) behavior^{8,9}. Based on these seemingly disparate observations, we sought to determine if adaptive immunity also fostered malignancy in the breast by regulating the phenotype or effector functions of tumor-associated macrophages (TAMs) and either activated their pro-tumor properties or alternatively by suppressing their anti-tumor capabilities.

Utilizing the MMTV-PyMT mouse model of mammary carcinogenesis¹⁰, we revealed a provocative role for CD4⁺ T cells as potentiators of peripheral blood dissemination and pulmonary metastasis of malignant mammary adenocarcinomas through their ability to regulate pro-tumor properties of TAMs¹¹. Specifically, T_H2-polarized CD4⁺ T cells secrete high levels of interleukin (IL)-4 and thereby regulate M1 and M2-type TAM bioactivity by activation of IL4R α -signaling cascades. M2-TAMs in turn promote invasive behavior of malignant MECs by high level production of cathepsin protease activity⁷ and EGF that subsequently activates MEC invasion and EGF receptor signaling programs, activities that are essential for entry into peripheral blood, dissemination and outgrowth in the lung. These findings indicate that when CD4⁺ T lymphocytes are present in a T_H2-type tumor microenvironment, they promote metastasis by regulating the pro-tumor properties of TAMs mediated by IL4R α -signaling, as opposed to limiting or eradicating malignant cells by engaging cytotoxic mechanisms. These realizations provide rational for development of anti-cancer therapeutics that neutralize pro-tumor properties of IL4R α -based signaling in both adaptive and innate immune cells in the tumor microenvironment and periphery, that when delivered in combination with cytotoxic drugs or therapeutics bolstering anti-tumor immunity, may extend survival of patients with advanced disease.

Leukocytes and Squamous Carcinogenesis: B lymphocytes constitute a central component of adaptive immunity and not only serve in antibody production but also as antigen-presenting cell; thus, B lymphocyte expression of major histocompatibility complex (MHC) and co-stimulatory molecules as well as secretion of pro-inflammatory cytokines induces optimal CD4⁺ and CD8⁺ T cells activation,

expansion, memory T lymphocyte formation and antigenic spreading. As such, B cells have been historically associated with anti-tumor immunity. More recently, the heterogeneity of B lymphocyte responses has been recognized and diverse B cell subtypes with either pro-immune or regulatory properties have been identified *in vivo*. In particular, regulatory B lymphocytes, which include various flavors of IL-10 producing cells, have been identified in the context of autoimmune diseases that exert anti-inflammatory activities^{12,13}. However, the role of these individual B lymphocyte subpopulations in malignant disease has yet to be fully elucidated.

Using a transgenic mouse model of multi-stage epithelial carcinogenesis, *i.e.*, K14-HPV16 mice¹⁴, we previously revealed that adaptive immunity is an important regulator of inflammation-associated cancer development¹⁵. Combined B and T lymphocyte-deficiency in HPV16 mice, *e.g.* HPV16/RAG1^{-/-} mice, resulted in a failure to initiate and/or sustain leukocyte infiltration during premalignancy¹⁵. As a consequence, tissue remodeling, angiogenesis and epithelial hyperproliferation were significantly reduced, culminating in attenuated premalignant progression and a 43% reduction in carcinoma incidence¹⁵. Importantly, adoptive transfer of B lymphocytes or serum from HPV16 mice into HPV16/RAG1^{-/-} mice reinstated chronic inflammation in premalignant tissues, indicating that B cell-derived soluble mediators were necessary to potentiate malignant progression. More recently, we revealed that B cell-derived IgGs regulate neoplastic progression and subsequent carcinoma development by engagement of Fcγ receptors (FcγR) expressed on resident and recruited immune cells¹⁶. Specifically, we found that immune complex (IC)-stimulation of leukocyte FcRγ is critical for establishing a pro-tumor microenvironment in premalignant tissue that directs not only recruitment of leukocytes from peripheral blood, but also leukocyte composition, phenotype and bioeffector functions once within neoplastic tissue. As such, proangiogenic and protumorigenic functions of mast cells and macrophages are differentially regulated by humoral immunity and functionally contribute to squamous carcinogenesis. These findings have broad clinical implications as they reveal critical signaling pathways regulated by humoral immunity and FcRγ to target therapeutically in patients suffering from chronic inflammatory diseases at risk for cancer, as well as individuals harboring premalignant lesions where chronic inflammation compromises tissue integrity and enhances risk of malignancy.

Summary: While many factors regulate leukocyte propensity to either promote or repress tumor development, in tissue- and/or oncogene-specific manners, polarized T_H2-type adaptive immune responses foster pro- as opposed to anti- tumor programming of myeloid cells that in turn directly regulate many of the “hallmarks” of solid tumor development¹⁷. Neutralizing these various “pro-tumor” regulatory pathways may provide relief for some aspects of late-stage cancer development as monotherapy, but more likely when combined with cytotoxic-, targeted- and/or immuno-therapy will provide a survival advantage by bolstering induction of anti-tumor bioactivities of tumor-associated leukocytes that extend efficacy of therapy.

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LISA M. COUSSENS, PH.D.

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REGULATION OF PROTUMOR IMMUNITY AND CANCER DEVELOPMENT**LISA M. COUSSENS, PH.D.**

Department of Pathology and Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA 94143, USA. Email: Lisa.Coussens@ucsf.edu

Clinical and experimental studies have established that chronic infiltration of neoplastic tissue by leukocytes, i.e., chronic inflammation, promotes development and/or progression of various solid tumors [1, 2]; however, the organ-specific cellular and molecular programs that favor pro-tumor, as opposed to anti-tumor immunity by leukocytes are incompletely understood. While some leukocytes certainly exhibit anti-tumor activity, i.e., cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells [3-5], other leukocytes, most notably mast cells, CD4⁺ T cells, B lymphocytes, dendritic cells (DCs), granulocytes, immature monocytes and macrophages exhibit more bipolar roles, by virtue of their capacity to either hinder or potentiate tumor progression [1, 2]. A major question regarding these disparate leukocytes bioactivities is the degree to which their various pro- or anti-cancer activities are regulated by tissue-specificity and/or are responsive to individual oncogenic or tumor suppressive gene programming in early neoplastic tissue.

Leukocytes and Breast Carcinogenesis: In the breast, cancer development is in part characterized by a significant increase in the presence of both innate and adaptive immune cells, with B and T lymphocytes as well as macrophages representing the most abundant leukocytes present in neoplastic stroma [6]. Retrospective clinical studies examining identity of leukocytes in human breast cancer have revealed that high immunoglobulin (Ig) levels in tumor stroma (and serum), and increased presence of extra follicular B cells, T regulatory (T_{reg}) cells, high ratios of CD4/CD8 or T_H2/T_H1 T lymphocytes in primary tumors or in draining lymph nodes correlates with tumor grade, stage and overall patient survival [7-11]; thus, some facets of adaptive immunity may indeed be involved in fostering cancer development.

On the other hand, experimental studies have demonstrated that macrophages in primary mammary adenocarcinomas regulate late-stage carcinogenesis by virtue of their pro-angiogenic properties [12, 13], as well as fostering pulmonary metastasis by providing epidermal growth factor (EGF) and cathepsin proteases [14] to malignant mammary epithelial cells (MECs) and thereby enhancing their invasive (and metastatic) behavior [15, 16]. Based on these seemingly disparate observations, we sought to determine if adaptive immunity also fostered malignancy in the breast by regulating the phenotype or effector functions of tumor-associated macrophages (TAMs) and either activated their pro-tumor properties or alternatively by suppressing their anti-tumor capabilities.

Utilizing the MMTV-PyMT mouse model of mammary carcinogenesis [17], we revealed a provocative and functional role for CD4⁺ T effector cells as potentiators of peripheral blood dissemination and pulmonary metastasis of malignant mammary adenocarcinomas through their ability to regulate the pro-tumor properties of TAMs [18]. Specifically, T_H2-polarized CD4⁺ T lymphocytes secrete high levels of interleukin (IL)-4 and thereby regulate M1 and M2-type TAM bioactivity by activation of IL4R⁺ -signaling cascades. M2-TAMs in turn promote invasive behavior of malignant MECs by high level production of cathepsin protease activity [14] and

EGF that subsequently activates MEC invasion and EGF receptor signaling programs, activities that are essential for entry into peripheral blood, dissemination and outgrowth in the lung. These findings indicate that when CD4⁺ T lymphocytes are present in a T_H2-type tumor microenvironment, they promote metastasis by regulating the pro-tumor properties of TAMs mediated by IL4R⁻ signaling, as opposed to limiting or eradicating malignant cells by engaging cytotoxic mechanisms. These provocative realizations provide rationale for development of anti-cancer therapeutics that neutralize the pro-tumor properties of IL4R⁻ based signaling in both adaptive and innate immune cells in the tumor microenvironment and periphery, that when delivered in combination with cytotoxic drugs or therapeutics bolstering anti-tumor immunity, may thereby extend survival of cancer patients with advanced disease.

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Specifically, we found that that immune complex (IC)-stimulation of leukocyte FcR γ is critical for establishing a pro-tumor microenvironment in premalignant tissue that directs not only recruitment of leukocytes from peripheral blood, but also leukocyte composition, phenotype and bioeffector functions once within neoplastic tissue. As such, proangiogenic and protumorigenic functions of mast cells and macrophages are differentially regulated by humoral immunity and functionally contribute to squamous carcinogenesis. These findings have broad clinical implications as they reveal critical signaling pathways regulated by humoral immunity and FcR γ to target therapeutically in patients at risk for cancer development, *e.g.*, patients suffering from chronic inflammatory diseases, as well as individuals harboring premalignant lesions where chronic inflammation compromises tissue integrity and enhances risk of malignancy.

Summary: While many factors regulate the propensity of leukocytes to promote or repress primary tumor development and metastasis, some of which may be tissue- and/or oncogene-specific, polarized T_H2-type adaptive immune responses by B and T lymphocytes foster pro- as opposed to anti- tumor programming of myeloid cells that in turn directly regulate many of the “hallmarks” of solid tumor development [24]. Neutralizing these various “pro-tumor” regulatory pathways may provide relief for some aspects of late-stage cancer development as monotherapy, but more likely when combined with cytotoxic-, targeted- and/or immuno-therapy will provide a survival advantage by bolstering induction of anti-tumor bioactivities of tumor-associated leukocytes that extend efficacy of therapy.

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Lisa M. COUSSENS, Ph.D.

Cold Spring Harbor Laboratory, CSH NY USA

Inflammation and Cancer:

Polarized Immune Responses Regulate Cancer Development

Lisa M. C oussens. Department of Pathology, Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, 513 Parnassus Ave., HSW-450C San Francisco, CA 94143-0502 USA; Phone: 001-415-502-6378; e-mail: Lisa.Coussens@ucsf.edu

The concept that leukocytes are components of malignant tumors is not new; however, their functional involvement as promoting forces for tumor progression has only recently been appreciated. We are interested in understanding the molecular mechanisms that regulate leukocyte recruitment into neoplastic tissue and subsequent regulation those leukocytes exert on evolving cancer cells. By studying transgenic mouse models of skin, lung and breast cancer development, we have recently appreciated that adaptive leukocytes differentially regulate myeloid cell recruitment, activation, and behavior, by organ-dependent mechanisms. Thus, whereas premalignant progression, including chronic inflammation, activation of angiogenic programming, tissue remodeling and malignant conversion during skin carcinogenesis is B cell, Ig and Fc γ R-dependent, during mammary carcinogenesis by contrast, T_H2-polarized CD4⁺ T cells play a dominant role in regulating pro-tumor and pro-metastatic properties of M2-polarized macrophages and dendritic cells, that together regulate metastasis of malignant mammary epithelial cells to lung. To be presented will be recent insights into organ and tissue-specific regulation of epithelial cancer development by adaptive and innate immune cells, and thoughts on how these properties can be harnessed for effective anticancer therapeutics.

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**Targeting Macrophages as a Novel Therapeutic Approach for Malignant
Pleural Mesothelioma**

Nikita Kolhatkar^{1,3}, Magnus Johansson¹, Dario Barbone³, Adam Yagui-Beltrán²,
Raphael Bueno⁴, David J. Sugarbaker⁴, David M Jablons^{2,5}, V. Courtney Broaddus³,
Lisa M Coussens^{1,5}

Departments of ¹Pathology, ²Surgery, ³Lung Biology Center, and ⁵Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco. ⁴Division of Thoracic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts.

Mesothelioma is a life-threatening tumor, induced by inhalation of asbestos fibers, which is largely resistant to most chemotherapeutic approaches. One feasible approach could be to harness the power of the immune system to increase the chemosensitivity of mesotheliomas. Using a combination of immunohistochemistry and flow cytometry to analyze the leukocyte compositions of human mesotheliomas, we have found that 1) epithelioid and mixed mesothelioma tumor subtypes have a higher degree of immune cell infiltration, when compared to sarcomatous tumors, and 2) mesothelioma tumors have large infiltrations of macrophages ($31 \pm 4.6\%$ of the inflammatory cell population [CD45⁺]). Indeed, the percentage of macrophages in mesotheliomas exceeded that found in other thoracic malignancies thus far evaluated (NSCLC cancer, 9% ; esophageal, 4%). In view of recent data indicating that macrophages can be targeted therapeutically to minimize some aspects of cancer development, we investigated whether macrophages could be targeted to enhance chemosensitivity of human mesotheliomas. To address this question, we adapted a 3-dimensional spheroid growth model, enabling heterotypic culture of mesothelioma cells with macrophages. We found that mesothelioma chemoresistance can be lowered by co-incubation with macrophages. However, the magnitude of the response was dictated by specific macrophage phenotype. Macrophage phenotype and bioactivity is modulated by Th1 versus Th2 cytokine exposure that in turn regulate either an M1 (IFN- γ & LPS) or M2 (IL-4) phenotype. M1-polarized macrophages increased the response of malignant mesothelioma spheroids to pro-apoptotic chemotherapeutic agents, such as TRAIL and gemcitabine. Furthermore, our preliminary data indicate that primary human tumor-associated macrophages, isolated from untreated malignant mesotheliomas, have similar pro-apoptotic effects when polarized with M1 cytokines, suggesting that cytokine re-polarization of macrophages in mesothelioma tumors to an M1 phenotype could augment therapeutic efficacy.

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