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14. ABSTRACT

The grant focuses on testing the hypothesis is that the success rate of active immunotherapy of advanced melanoma based on vaccines or adoptive transfer of MAA-specific T cells can be optimized by metabolic reprogramming of T cells from glycolytic energy production towards the use of fatty acid oxidation. As we published, the interstitial fluids of melanomas have low glucose (Glc) contents while free fatty acid (FA) species increase during tumor progression. $CD8^+$ T cells upon activation in the periphery switch to glycolytic energy production. Once $CD8^+$ T cells enter the Glc-depleted environment of melanomas, starvation drives their differentiation towards functional exhaustion and apoptosis, unless they switch towards the use of alternative nutrients, such as FAs, for energy and biomass production. Metabolism can be modified by drugs, such as fenofibrate (FF), an agonist of PPAR-α. This in turn improves tumor infiltrating lymphocyte (TIL) functions, which results in more sustained tumor regression. $CD8^+$ TIL performance can be further enhanced by complementing metabolic reprogramming with a PD-1 checkpoint inhibitor, which in melanoma renders PD- $L1^+$ tumors cells more susceptible to cytolysis. These hypotheses are supported by our data.[22] Most of these studies were thus far conducted in mice using adoptive transfer models. Prior to clinical trials, the relevance of our findings for human tumors has to be confirmed using approaches that are suitable for use in melanoma patients.

15. SUBJECT TERMS
Cancer vaccine, mouse model, melanoma, $CD8^+$ T cells, metabolism, PPARa agonist, checkpoint blockade, human melanoma samples, iPDX model, NOD-SCID mice, human tumor transplantation, adoptive lymphocyte transfer.

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1. INTRODUCTION:

The grant focuses on testing the hypothesis is that the success rate of active immunotherapy of advanced melanoma based on vaccines or adoptive transfer of MAA-specific T cells can be optimized by metabolic reprogramming of T cells from glycolytic energy production towards the use of fatty acid oxidation. As we published, the interstitial fluids of melanomas have low glucose (Glc) contents while free fatty acid (FA) species increase during tumor progression. $CD8^+$ T cells upon activation in the periphery switch to glycolytic energy production. Once $CD8^+$ T cells enter the Glc-depleted environment of melanomas, starvation drives their differentiation towards functional exhaustion and apoptosis, unless they switch towards the use of alternative nutrients, such as FAs, for energy and biomass production. Metabolism can be modified by drugs, such as fenofibrate (FF), an agonist of PPAR-□. This in turn improves tumor infiltrating lymphocyte (TIL) functions, which results in more sustained tumor regression. $CD8^+$ TIL performance can be further enhanced by complementing metabolic reprogramming with a PD-1 checkpoint inhibitor, which in melanoma renders PD- $L1^+$ tumors cells more susceptible to cytolysis. These hypotheses are supported by our data. Year 1 focused on mouse studies, some of which were finished in year 2. In year 2 we started studies with human melanoma samples.

2. KEYWORDS:

Cancer vaccine, mouse model, melanoma, $CD8^+$ T cells, metabolism, PPARa agonist, checkpoint blockade, human melanoma samples, iPDX model, NOD-SCID mice, human tumor transplantation, adoptive lymphocyte transfer.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Tasks 1 and 2 focus on a mouse model of melanoma. In task 1, subtask 1 we were to gain IACUC and ACURO approval for the proposed animal experiments of tasks 1 and 2. This was achieved. In subtask 2 we were to determine tumor progression and characteristics of $CD8^+$ T cells induced by a melanoma vaccine comparing treatment with a PPARα agonist (fenofibrate) to diluent (DMSO) treatment. In task 2 fenofibrate treatment was to be combined with PD-1 checkpoint blockade again monitoring tumor progression and characteristics of vaccine induced T cells. The goal of tasks 1 and 2 was to show if the PPARα agonist given directly to tumor-bearing mice slows tumor progression and if this is linked improved frequencies and functions of vaccine-induced TILs, changes in TIL metabolism and/or delayed TIL exhaustion. Timeline: Year 1 of the award
Tasks 3 and 4 focus on human TILs from melanoma metastases tested in severely immunodeficient mice carrying tumor fragments from the same patients. In subtask 1 we are to gain IRB approval for subtasks 3 and 4. This has been obtained. We have submitted an animal protocol and are awaiting

IACUC approval. Subtask 2 will determine if melanoma cells from human metastasis express PD-L1. In subtask 3 we will isolate and analyze lymphocytic infiltrates from the tumors. Subtask 4 will inject melanoma fragments into NOD/SCID mice, which will be treated with fenofibrate or diluent. Once tumors reach a certain size lymphocytes will be isolated and characterized for expression of differentiation markers and transcripts involved in glucose and fatty acid metabolism. In subtask 5 we will take the same approach but combine fenofibrate treatment with PD-1 checkpoint blockade. Task 4 will determine the effects of metabolic reprogramming and PD-1 treatment on adoptively transferred human $CD8^+$ T cells. Subtasks 1 and 2 will expand human melanomas in NOD/SCID mice and thereafter determine if the tumor cells express PD-L1. Subtasks 3- 5 will expand and then cryopreserve T cell from the same tumors or from matching PBMC samples. Once tumors have expanded in the NOD/SCID mice, lymphocytes will be thawed and cultured with diluent or fenofibrate. They will then in subtask 7 be infused into NOD/SCID mice bearing autologous tumors. Mice will be treated with fenofibrate or diluent. Tumor progression will be monitored and after euthanasia human TILs will be characterized. Subtask 8 will take the same approach but include a PD-1 checkpoint inhibitor into the treatment regimen.
The goal of the experiments of Task 3 is to determine if the PPARα agonist treatment with or without PD-1 checkpoint blockade affects human melanoma progression and characteristics of the tumors' lymphocytic infiltrate. The goal of task 4 is similar but will used adaptively transferred lymphocytes that were expanded in vitro rather TILs that are present within the transplanted tumor fragments.
Timeline: Task 3 year 2 of the award; Task 4 year 3 of the award.

What was accomplished under these goals?

Research Accomplishments

Task 1 and 2: Effects of metabolic reprogramming without or combined with PD-1 blockade on mouse TILs. The original aim was to combine vaccination and metabolic reprogramming with PD-1 checkpoint blockade.

We finished the experiments of task 2 by analyzing the transcriptome of $CD8^+$ TILs from the following 4 groups of vaccinated mice:
Group 1 was treated with DMSO and an isotype control antibody
Group 2 was treated with DMSO and an anti-PD-1 antibody
Group 3 was treated with fenofibrate (FF) and an isotype control antibody
Group 4 was treated with FF and an anti-PD1 control antibody
$CD8^+$ TILs were tested by a quantitative RT-PCR for transcripts encoding factors involved in glucose and fatty acid metabolism under the expectation that FF would increase fatty acid catabolism and reduce glucose metabolism while PD-1 blockade would have the opposite effect.

This was in part supported by our results shown in Figures 1A and 1B. Figure 1A shows the cycle threshold value (CT) while 1B shows the fold difference comparing samples from mice treated with DMSO or FF comparing those that in addition received the isotype control antibody to those treated with PD-1.

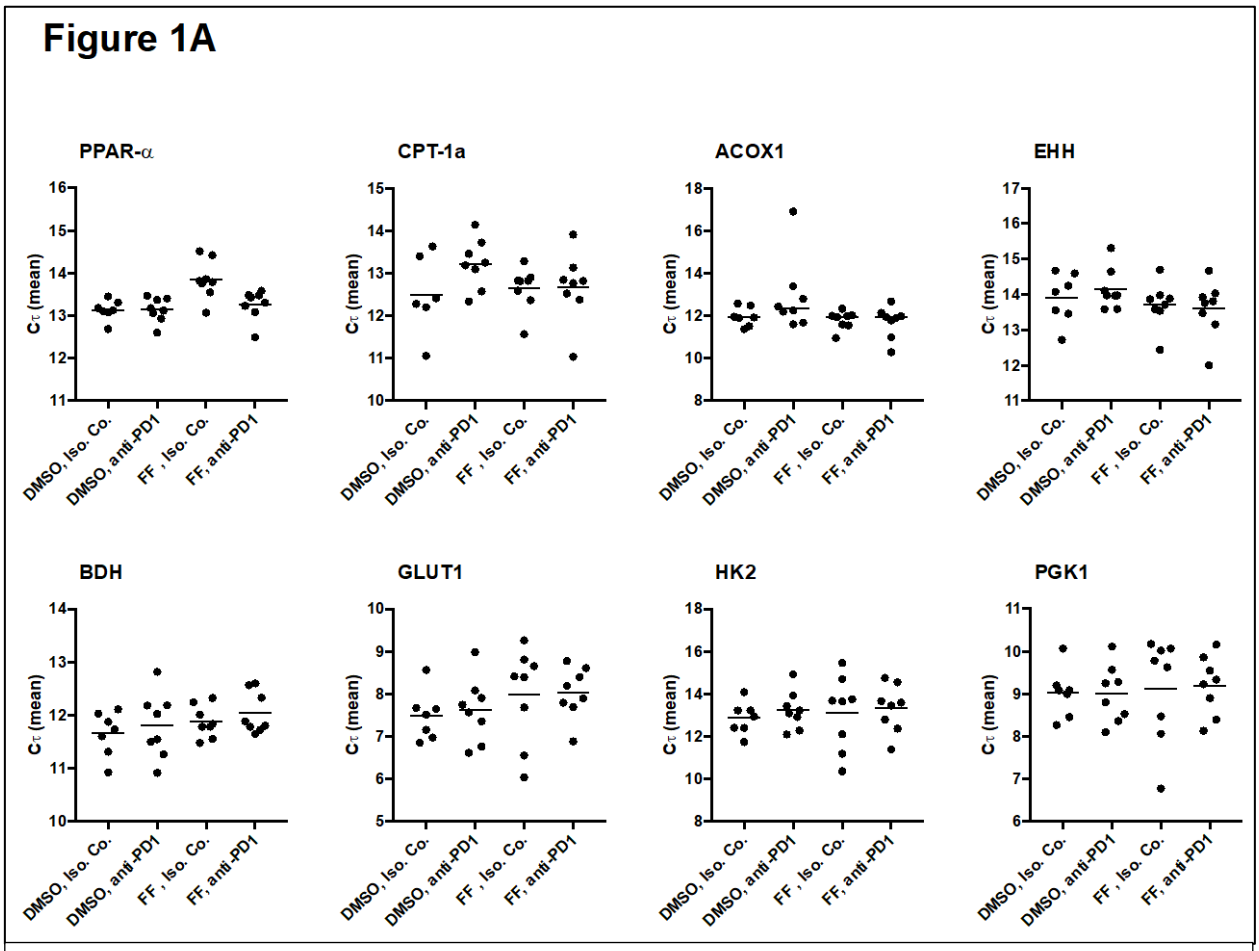


Figure 1A shows Ct values for individual $CD44^{+}CD8^{+}$ samples from tumors of fenofibrate or DMSO treated mice.

Figure 1A shows modest differences for the following groups. PPARa was decreased in the FF, isotype control group and this effect of FF was not seen in mice that also received the anti-PD-1 antibody. The anti-PD-1 antibody caused in the DMSO but not in the FF group a slight but consistently decrease in many of the transcripts encoding enzymes of fatty acid catabolism such as CPT-1a, ACOX1, EHH and BDH. The most pronounced change was seen for GLUT-1 whose expression decreased in the FF groups.

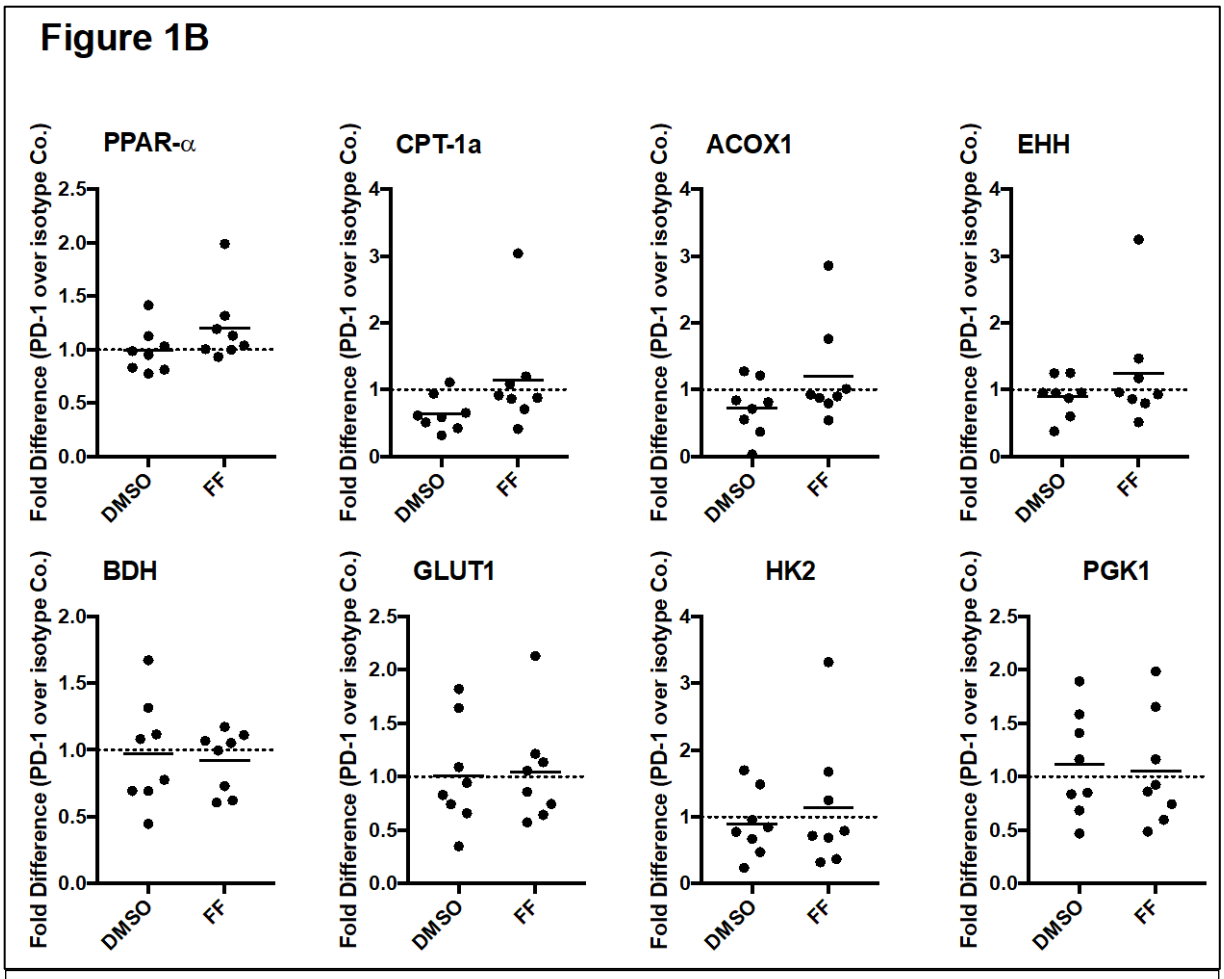


Figure 1B shows fold differences for individual $CD44^{+}CD8^{+}$ samples from tumors of anti-PD-1 treated mice in comparison to averages of the isotype control antibody treated

Comparing the effects of PD-1 blockade given alone (i.e., DMSO group) or together with FF showed that the anti-PD-1 antibody differentially increased expression of PPAR-a in the FF but not the DMSO group while vice versa effects of the anti-PD-1 antibody on reduction of transcripts for CPT-1a and ACOX1 were mainly seen in the DMSO group. Overall, these results were expected.

Figure 2

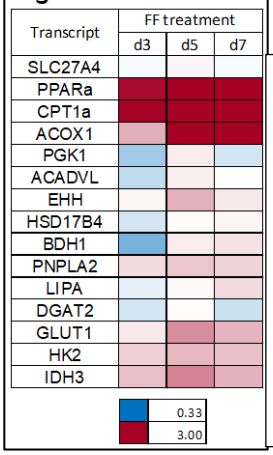


Figure 2 shows as a treatment the effect of FF treatment initiated on different days after vaccination on the indicated transcript levels, red indicates increases and blue decreases after FF treatment (see legend).

As we reported in our 1st annual progress report, we changed FF treatment after first testing if and how onset of treatment in relation to vaccination affects $CD8^+$ TIL metabolism. As we reported the effects were more pronounced when treatment was delayed till day 5 rather than day 3, which we had used for adoptive transfer studies. It was remarkable though that FF, a PPARa agonist that is known to increase fatty acid metabolism also caused if given 5 or 7 days after vaccination a robust increase in the expression of GLUT1, the major receptor for glucose uptake (Figure 2) and HK2 the 1st rate limiting enzyme of glycolysis.

Metabolism is driven by the availability of nutrients and these data suggest that FF may increase glucose content within tumors potentially by increasing the use of fatty acids by tumor and stroma cells thus reducing consumption of glucose.

Figure 3

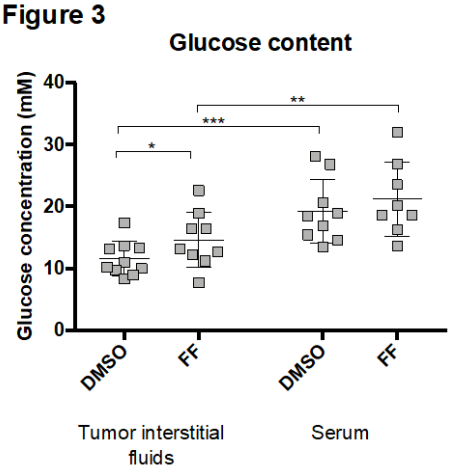


Figure 3 shows the glucose content within tumors and sera in individual FF or DMSO treatment mice.
Lines with stars above indicate significant differences by multiple t-tests (*) between cells from mice treated with FF or the diluent; p-value between 0.01-0.05, (**) p-value between 0.001-0.001, (***) p-value between 0.0001-0.001, (****) p-value < 0.0001.

This would enable T cells, which can normally not compete with tumor cells for glucose, to switch back at least in part to this key nutrient. We harvested tumors (~ 1.5 cm in diameter) from mice that had not been vaccinated but treated with DMSO or FF 8 days after challenge (mirroring the post day 5 schedule in vaccinated mice) and collected the tumor interstitial fluids. We collected sera from the same mice. Glucose content was measured with a glucose meter. As shown in Figure 3 there was a significant increase in glucose within tumors of mice that received FF compared to those that were treated with DMSO. We assume that this reflects that FF increased fatty acid metabolism by tumor and tumor stroma cells thus reducing glucose consumption, which in turn made this nutrient more available to infiltrating T cells.

To test this further, we isolated RNA from the tumors of the same groups of mice, which had not been vaccinated but had been treated with FF or DMSO and upon FF treatment showed increased levels of glucose in the tumor interstitial fluids. Melanomas of mice that are not vaccinated have low contest of T cell infiltrates and we therefore assume that the cellular composition reflects mainly tumor cells, tumor stroma cells and subsets of myeloid cells. Upon reverse transcription we tested the cDNA for transcripts of fatty acid metabolism, i.e., PPARa, CPT1a and ACOX1 and for transcripts encoding factors of glucose metabolism, i.e., GLUT1, HK2 and PGK1. In most mice transcripts for PPARa and ACOX1 increased upon FF treatment while transcripts encoding the glucose receptors or enzymes of glycolysis decreased again indicating that FF not only affect

metabolism of vaccine-induced T cells but also the metabolism of other cells within the tumors (Fig. 4).

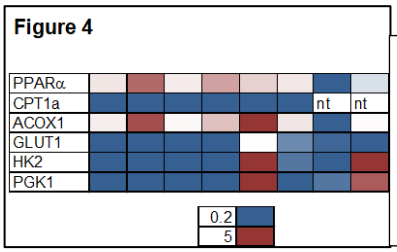


Figure 4 shows as heatmap the effect of FF treatment initiated on day 8 after challenge on the indicated transcript levels within tumors. Red indicates increases and blue decreases after FF treatment. Intensity of colors reflects magnitude of differences.

We submitted a manuscript describing our results of task 1 to Cancer Research and they requested additional experiments.

1. The reviewers requested that we explore the effect of fenofibrate on glucose uptake by TILs. We isolated TIL from tumor bearing vaccinated mice treated with FF or DMSO and tested their glucose uptake using 2NBDG, a fluorescent analogue of glucose, directly ex vivo. Cells were stained with various leukocyte markers and the then analyzed by flow cytometry. As shown in Figure 5 T cells subsets isolated from tumors of fenofibrate treated mice showed increased glucose uptake compared to those from DMSO treated mice. This difference was not observed for tumor-infiltrating myeloid cells or neutrophils.

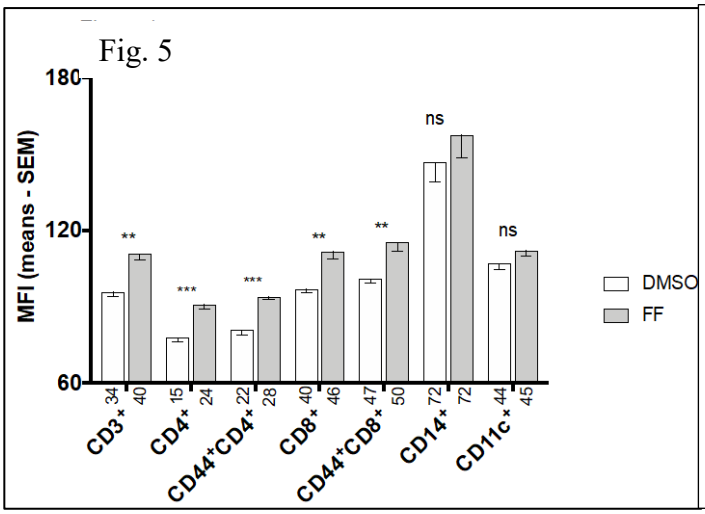


Figure 5. The graph shows the mean fluorescent intensity of 2NBDG in various TIL populations for DMSO (white bars) or fenofibrate (grey bars) treated mice identified by stains for CD3, CD8, CD4, CD44, CD14 and CD11c. The. numbers below the bars show percentages of dye$^+$ cells. Stars above the bars indicate significant differences: (**) p-value between 0.001- 0.01, (***) p-value between 0.0001- 0.001 by multiple t-tests.
ns – not significant

2. We were asked to test if fenofibrate could induce a melanoma-specific CD8$^+$ T cell response in unvaccinated tumor-bearing mice or change the phenotypes of activated splenocytes or TILs or if these changes were specific for vaccine-induced T cells. Mice were either given AdC68-gDMelapoly or the diluent and then 5 days later they were treated daily for 3 weeks with fenofibrate (Fig 6A). Splenocytes and TILs were isolated 1 day after treatment was completed and tested by tetramer-staining for frequencies of CD8$^+$ T cells to the immunodominant epitope of Trp-1. Fenofibrate given without vaccination did not induce such as response (Fig. 6B). To assess phenotypes, we compared naïve and recently activated, i.e., PD1^{hi}CD62L^{low} CD8+ T cells from the unvaccinated mice to tet$^+$ or tet$^+$ PD1^{hi}CD62L^{low} CD8$^+$ T cells from the vaccinated group from spleens and tumors. Activated CD8$^+$ T cells from unvaccinated mice had after fenofibrate treatment similar percentages of cells with high levels for Tbet, CD127, LAG-3 and HIF-1a and only slightly lower frequencies for PPARa$^+$ cells indicating that the drug independent of the vaccine affected

these changes in all activated $CD8^+$ T cells but not in naïve T cells. Differences were mainly seen for KLRG1 which was only high on tet^+CD8^+T cells from the vaccine group (Fig. 6C).

Fig. 6

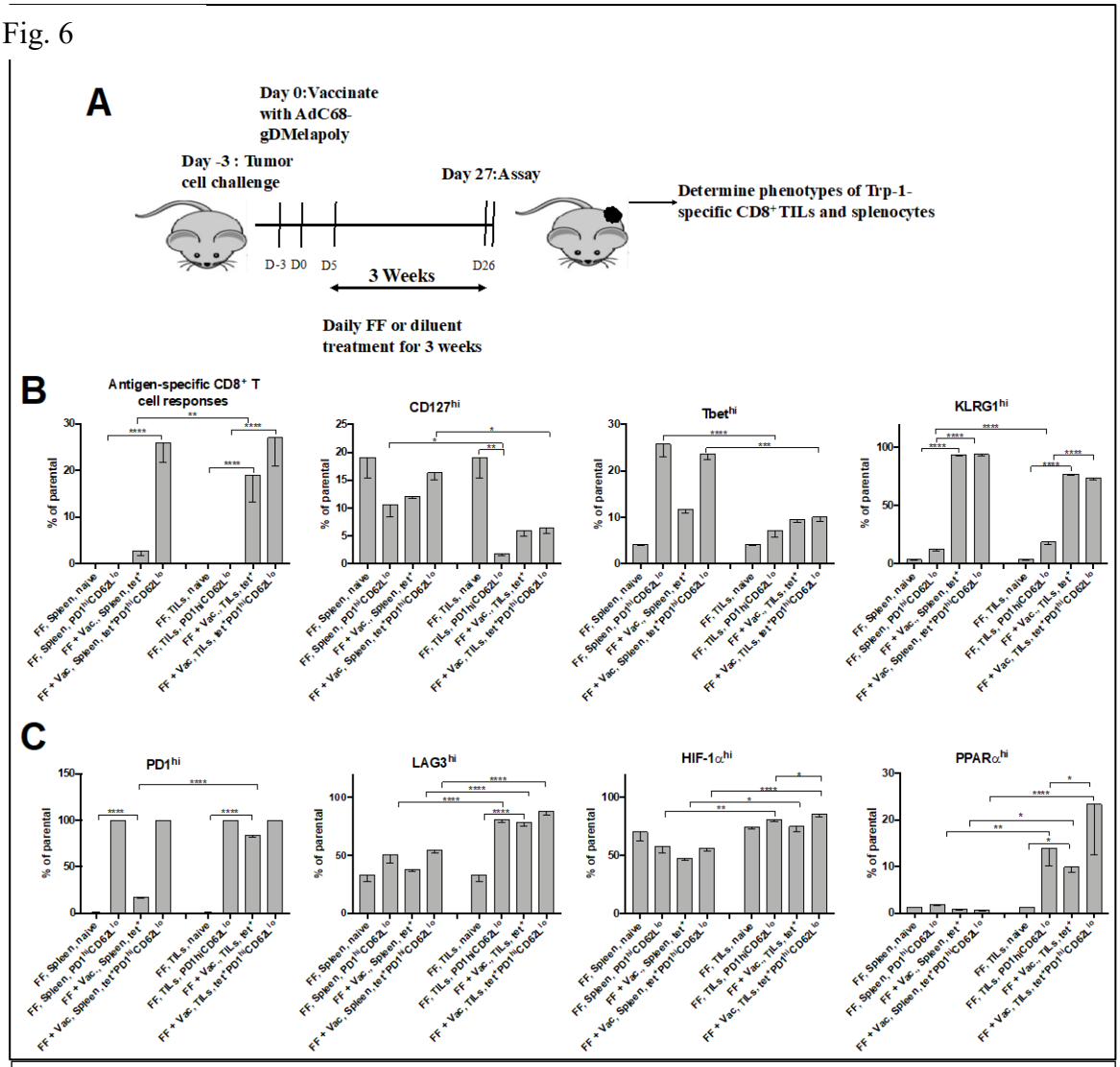


Figure 6. A The cartoon describes the experimental set-up. B shows frequencies of Trp-1-tet^+ $CD8^+$ in spleens and tumors. To better compare cells from naïve and vaccinated mice we gated on naïve cells (CD62Lhi), recently activated cells ($PD1^{high}$,$CD62L^{low}$), $tetramer^+CD8^+$ T cells over all CD8 T cells or $tetramer^+$ $CD8^+$ T cells over all recently activated $PD1^{high}$,$CD62L^{low}$ $CD8^+$ T cells. C shows phenotypes

The reviewers asked us to analyze tumors from vaccinated DMSO or fenofibrate treated mice by immunohistochemistry for differences in T cell infiltrates. Mice were challenged with tumor 4 µm sections of paraffin embedded tumors were mounted on charged slides and baked in a 60^0 C oven for 1 hr. Tissues were deparaffinized and rehydrated in 2 changes of Xylene substitute, 2 changes of 100% EtOH, 2 changes of 95% EtOH, and 2 changes of DI water. Then hydrophobic barriers were drawn around the tissue sections with Vector ImmEdge Pen. Antigen retrieval was achieved with

DAKO EDT (pH 9) under pressure at 110^{0}C for 10 min. Slides were cooled on the counter for 20 min, then rinsed in DI water. After that, they were immersed in a 3% hydrogen peroxide solution for 10 min, then rinsed in DI water. First primary antibody (Anti-CD8 and anti-CD4 from Cell Signaling Technology) was applied to the bottom tissue section. Antibody is diluted with Cell Signaling Tech. Signal Stain Ab diluent. Top section serving as negative control is covered with TBST. Overnight incubation at 4^{0}C. After rinsing, secondary antibody (Vector HRP anti-rabbit polymer) was applied to all sections and incubated for 30 min at room temp. AEC (red) Vector peroxidase substrate (CD4) and second chromagen, Abcam Stay Blue (CD8) were applied and incubated for 30 min and 10 min respectively.

Sections were analyzed with a Nikon Eclipse Ti Inverted Microscope, Light Engine SOLA SE II 365, PSF (Perfect Focus System), Motorized FL Filter Turret, Prior Stage, Piezo Stage, using a Nikon 20X Plan Apo, N.A. .95 objective and the Nikon NIS Elements AR Version 5.30.02 (Build 1545) software. Nis Elements Ar (Scan Large Image) defined two regions of interest, size 8 × 8 fields, total 88-128 frames (2088 × 2048 px) in X & Y per section, with no overlap between images. Images were taken with a Nikon Fi3 color camera (2048 × 2048-pixel resolution). Numbers of CD4$^+$ and CD8$^+$ T cells were higher within tumors of fenofibrate than DMSO-treated mice which likely reflects increased recruitment, retention, or survival Figure 7).

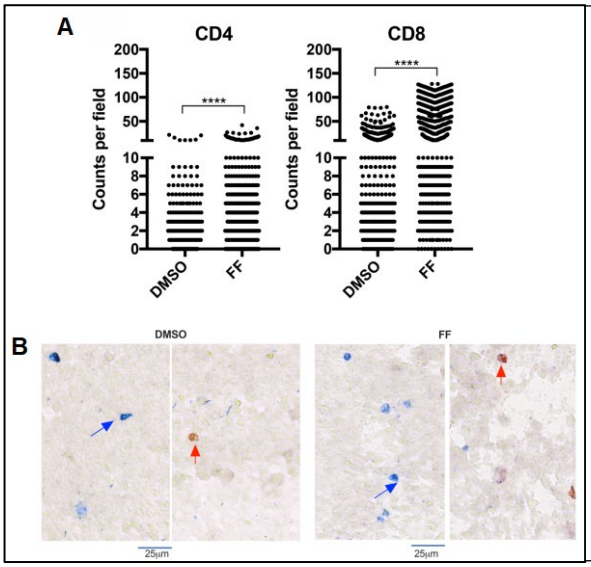


Figure 7. [A] shows results from the analyses of histograms from tumor sections of the two groups of mice. The graph shows numbers of cells per field. Sections from 5 fenofibrate treated and 3 DMSO treated mice were analyzed. In each section cells were counted from 88-128 fields. Differences between the groups were determined by student t-test. CD4$^+$ T cells – p-value: 2.1E-08, CD8$^+$ T cells – p-value: 4.8E-27. [B] shows CD4$^+$ and CD8$^+$ T cells within tumors of vaccinated mice that were treated with DMSO (3 mice) or fenofibrate (5 mice), examples for CD8$^+$ cells and CD4$^+$ T cells are highlighted by blue or red arrows, respectively.

Task 3: Effects of metabolic reprogramming on human TILs. We have thus far focused on task 3 using the iPDX model. We obtained institutional and DoD approval to conduct experiments with materials from human subjects in mice. The facility at the University of Pennsylvania that provides melanoma samples to investigators was closed for many months due to the COVID-19 pandemic but eventually reopened.

Table 1 described the tumor samples that have been transplanted, their growth characteristics and the type of analyses we conducted.

We have thus far obtained 7 tumor samples that were transplanted into mice: (1) three primary melanomas, (2) one Merkel cell carcinoma, (3) two recurrent melanomas with one of those from a patient on nivolumab and 2 melanoma metastases. Most tumors have thus far not yet grown in

mice, or they are growing very slowly. Interesting the one tumor that grew very fast (group E) was from a patient with a recurrent melanoma, who was being treated with nivolumab, a monoclonal antibody directed against PD1.

Table 1

Mice (ID)	Group	Treatment	Tumor growth	Tumor type	Treatment	Analysis
1	Group A	FF	no growth	Primary melanoma	3 mo after transplant for 21 days	Phenotyping blood (3,5 mo)
2		FF	no growth			
3		FF	no growth			
4		FF	no growth			
5		FF	dead			
6	Group B	DMSO	growth (slow)	Merkel cells carcinoma	2 mo after transplant for 21 days	Phenotyping blood (2, 4 mo)
7		DMSO	growth (slow)			
8		DMSO	no growth			
9		DMSO	no growth			
11	GroupC	FF	growth, euthanized	Recurrent melanoma	1 week after transplant for 21 days	2 mice (sick) phenotyping TILs
12		FF	growth, euthanized			
13		FF	growth (slow)			Phenotyping blood (1, 3 mo)
14		DMSO	no growth			
15	Group D		growth (slow)	Primary melanoma		Phenotyping blood (2mo)
16			growth (slow)			
17			growth (slow)			
18			growth (slow)			
19			died			
20	Group E	DMSO	growth (fast)	Recurrent melanoma under nivolumab	2 mo after transplant for 21 days	Phenotyping blood (2, 3 mo), mice euthanized, tumor progression
21		DMSO	growth (fast)			
22		FF	growth (fast)			
23		FF	growth (fast)			
24	Group F		so far no growth	Primary melanoma		
25			growth (slow)			
26			so far no growth			
27			so far no growth			
28			so far no growth			
29	Group G		new implants	Metastatic melanoma		
30			new implants			
31			new implants			
32			new implants			
33			new implants			
34	Group H		new implants	Metastatic melanoma		
35			new implants			
36			new implants			
37			new implants			
38			new implants			

Once tumors from this patient transplanted into group E (Table 1) reached a diameter of ~ 1 cm we started the mice on either fenofibrate or DMSO and monitored tumor progression. As seen in Figure 8A, with the caveat that each group had only two mice, treatment with fenofibrate appeared to slow tumor progression.

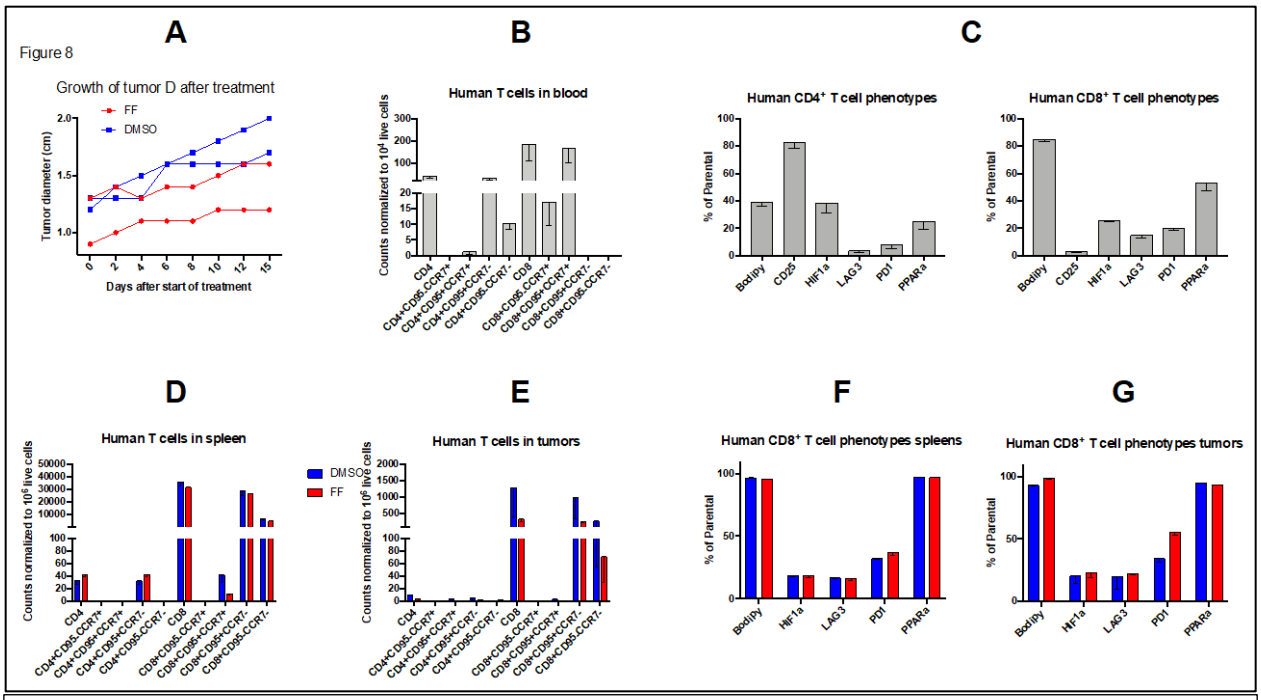
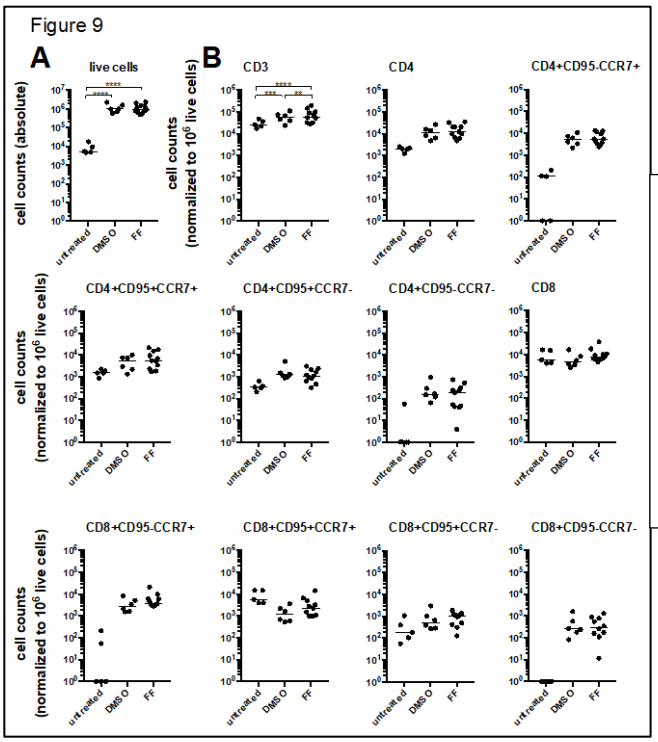


Figure 8. [A] shows tumor progression over time. Day 0 reflects onset of drug treatment. B shows normalized numbers of human T cell subsets that were recovered from blood. C shows phenotypes of circulating human CD4+ and CD8+ T cells. D shows normalized human T cell counts in spleens and (E) in tumors. F shows phenotypes of T cells from spleens or tumors (G).

Mice of group E were bled 6 weeks after they received the tumor transplants and PBMCs were analyzed for presence and phenotypes of human T cells. As shown in Figure 8B CD4+ and CD8+ T cells were detected in blood. CD8+ T cells were more frequent than CD4+ T cells. Most CD4+ T cells were CD95+CCR7- suggesting that they belonged to the effector memory cell subsets while most CD8+ T cells were double positive for CD95 and CCR7 suggestive of central memory or stem cell memory T cells. Phenotypically CD4+ T cells differed from CD8+ T cells. Higher percentages of CD4+ T cells were positive for CD25 while percentages of CD8+ T cells positive for Bodipy or exhaustion markers were higher. Mice were euthanized after drug treatment and lymphocyte recovery was analyzed from spleens (Fig. 8D) and tumors (Fig. 8E). By then human CD4+ T cells were virtually absent from spleens or tumors. CD8+ T cells in spleens and tumors mainly belonged to the effector memory subset. In tumors with the caveat that we only had 2 mice per group fenofibrate treatment reduced numbers of recovered CD8+ T cells. Phenotypically CD8+ T cells from spleens and tumors were similar. Treatment with fenofibrate increased percentages of PD-1+ CD8+ T cells but affected no other shifts in phenotypes.

We collected PBMC samples from several of the groups of mice as shown in Table 1. Some mice had been treated with DMSO; others been treated with fenofibrate. One group had not yet been treated.
The latter group received fragments of a primary melanoma that was apparently very poorly infiltrated for recovery of human CD3+ cells from blood of these mice was significantly lower that



from mice of the other groups (Figure 9A). Portions of circulating human CD3+ T cell were also lower; portions of other human lymphocyte populations were similar (Figure 9B).

Figure 9. A shows absolute numbers of live cells recovered from blood. B shows normalize counts for different T cell populations. Differences were determined by Fisher's LSD test. Significant differences are indicated by lines connecting the compared groups. Stars above the lines show level of significance.

Human T cell were phenotyped. Again, there were major differences between the untreated groups and the groups that had already undergone treatment. Significant differences between the two latter groups were seen for CD4+ T cells for PPARa and Bodipy; as expected fenofibrate treatment increased the percentages of CD4+ T cells positive for these two markers of fatty acid metabolism (Figure 10).

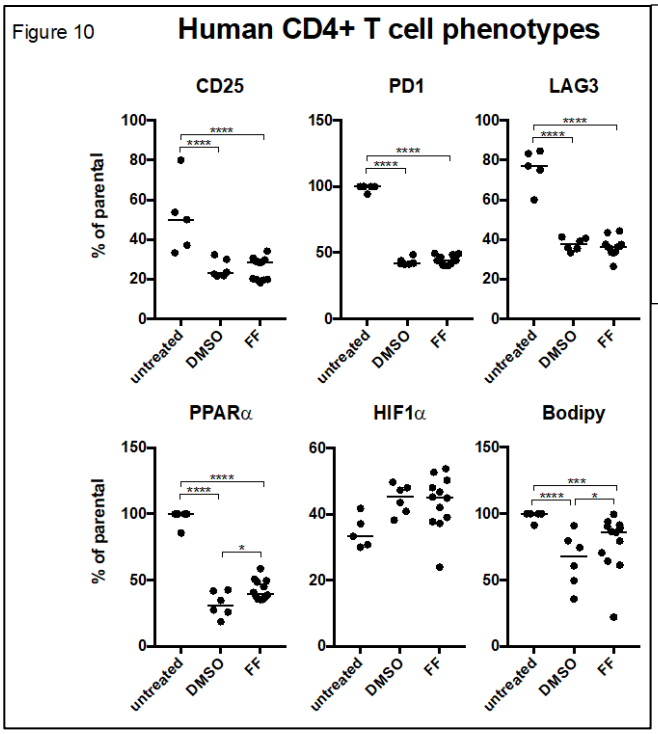


Figure 10 . A shows phenotypes of circulating human CD4+ T cells. Differences were determined by Fisher's LSD test. Significant differences are indicated by lines connecting the compared groups. Stars above the lines show level of significance.

Results were similar for human CD8+ T cells for which the difference between the DMSO and fenofibrate treated groups reached significance for Bodipy. There was a trend towards higher percentages of PPARa^{+} cells and HIF-1a^{-} cells which failed to reach significance. Overall, these results show that fenofibrate changed the metabolism of the tumor infiltrating T cells that left the tumors within the mice and circulated in blood.

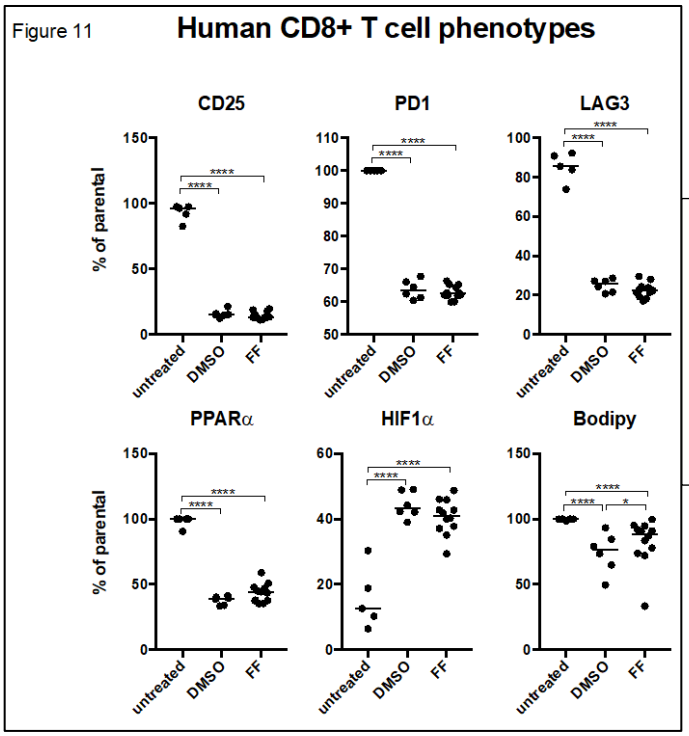


Figure 11. A shows phenotypes of circulating human CD8+ T cells. Differences were determined by Fisher's LSD test. Significant differences are indicated by lines connecting the compared groups. Stars above the lines show level of significance.

We analyzed human TILs from one tumor and two mouse spleens as 2 mice of group C became ill and had to be euthanized. Both mice had received tumor fragments, one was treated with fenofibrate the other with DMSO. The DMSO treated mouse carried a small tumor from which we isolated TILs, the fenofibrate treated mouse was tumor-free. Percentages of marker+ T cells but for HIF-1a on CD8+ T cells were higher on TILs than splenocytes (Figure 12).

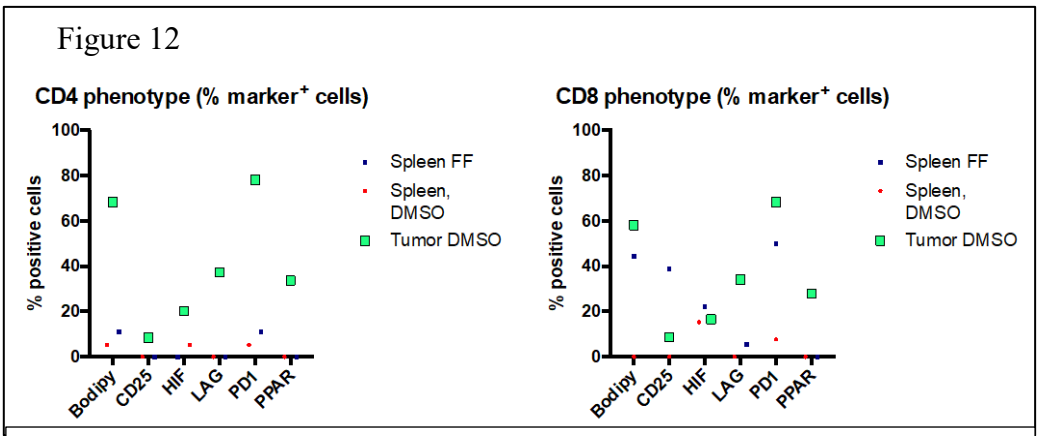


Figure 12 show % marker positive CD4^{+} (left) and CD8^{+} T cells (right) from a tumor or spleens of individual mice treated with DMSO (spleen blue, tumor green) or fenofibrate (spleen red).

What opportunities for training and professional development has the project provided?

Dr. A. Chekaoui was recruited as a postdoctoral fellow to this project. He has learned a number of techniques and has written a manuscript that was revised, and the revision is under review. He participated in training related activities at the Wistar Institute, including training in 'Ethical Research Standards". He provided training to a rotation graduate student.

How were the results disseminated to communities of interest?

We submitted a manuscript to Cancer Research. The reviewers requested additional experiments. We conducted the requested experiments, and the revised manuscript is now undergoing review

What do you plan to do during the next reporting period to accomplish the goals?

We are planning to revisit the PD-1 experiments of task 2 using the new schedule of fenofibrate treatment. As we show in the manuscript, fenofibrate given directly to tumor growing mice that are then vaccinated slows down tumor growth. The drug instructs tumor cells to increase fatty acid catabolism which leads to an increase of glycose within the tumor microenvironment. The glucose becomes available as a nutrient for T cells. PD-1 signaling increases fatty acid metabolism so blocking this signaling by an anti-PD-1 antibody is expected to increase glucose uptake by T cells and thereby to further increase their fitness.
In task 3 we will check phenotypes from one already available group of mice with growing tumors (group D). We will then switch to testing PBMCs and TILs for transcripts and start treating mice with an anti-PD-1 antibody as well.
We are starting with task 4. We will use parts of each tumor sample to finish task 3 experiments but from each tumor we will remove a section to grow tumor cells and expand TILs for the task 4 experiments.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

We show that combining the melanoma vaccine with fenofibrate treatment reduces tumor progression in mice. We determined the mechanism. Tumor cells and tumor stroma cells decrease glycolysis and increase fatty acid catabolism in fenofibrate treated mice. This in turn increases the overall glucose level within the tumor microenvironment making the nutrient available to TILs.

What was the impact on other disciplines?

The concept of metabolic manipulation of T cells may increase treatment efficacy in other diseases such as some chronic viral infections where T cells migrate to a microenvironment that is poor in nutrients.

What was the impact on technology transfer?

Wistar's business development team believes that the findings disclosed during the performance of this grant are novel, non-obvious and useful and will be used to support the filing of a new provisional patent application. Wistar is actively seeking a co-development partner to assist with the translation of these findings.

What was the impact on society beyond science and technology?

This would require confirmation of the mouse studies by clinical trials, which may inform if and what type of metabolic manipulations which can in theory not only be achieved by drugs but also by changes in diets or by changes in the microbiome would benefit patients with solid tumors.

5. CHANGES/PROBLEMS:

We made some minor technical adjustments but no significant changes.

Changes in approach and reasons for change

N/A

Actual or anticipated problems or delays and actions or plans to resolve them

Due to the COVID-19 pandemic the facility at the Hospital of the University of Pennsylvania was temporarily closed which caused a slight delay in our ability to obtain approval for the task 3 and 4 animal protocols (which required information that we needed to obtain from the facility. Starting this task thus has been delayed by several months. We had a case of COVID-19 in our laboratory which resulted in a 2 week quarantine for most of the laboratory members.

Changes that had a significant impact on expenditures

None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

None

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

None

6. PRODUCTS:

Nothing to report

- **Publications, conference papers, and presentations**

  Journal publications.

Review article: Zhang Y, Ertl HCJ. T and B cell Metabolism in Older Adults. Immunometabolism. 2020;2(3):e200001. https://doi.org/10.20900/immunometab20200001 Published Chekaoui A and Ertl HCJ. Fenofibrate, a PPARa agonist increases the effectiveness of a cancer vaccine to reduce tumor progression. Under review

Books or other non-periodical, one-time publications.

None

Other publications, conference papers and presentations.

None

- **Website(s) or other Internet site(s)**

None

- **Technologies or techniques**

Timing and dosing of fenofibrate in relation to vaccination. These data are shared in the submitted publication.

- **Inventions, patent applications, and/or licenses**

None

- **Other Products**

None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Areski Cherkaoui, PhD, Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 12

Contribution to Project: Dr. Areski performed the T cell assays, cell sorting and PCR studies
Funding Support: Fully supported on this award.

Name: Christina Cole, Lab Manager
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1.2

Contribution to Project: Christina Cole writes, submits and amends animal and human subject protocols and assists Dr. Ertl in assembling and submitting progress reports and manuscripts and in interactions with outside facilities such as the Core Facilities at the University of PA
Funding Support: Current award 1.2 cal; Virion 6.0 cal.; Mathers Foundation 4.8 cal.

Name: Hildegund Ertl, MD, PI
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 3.6

Contribution to Project: H. Ertl designs experiments, analyses data obtained by flow cytometry and qPCR, writes progress reports and manuscripts and oversees the efforts of the team
Funding Support: Current award 3.6 cal; Spark Therapeutics 0.25 cal; Virion 2.8 cal; Mathers Foundation 3.1 cal.; Oxford Rabies Collaboration .05 cal.; Institute/Other 2.2 cal

Name: Wynetta Giles-Davis, Research Technician
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 6

Contribution to Project: Wynetta Giles-Davis takes care of cell cultures, prepares media and orders supplies and animals
Funding Support: Current award 6 cal.; Virion 0.9 cal; Mathers Foundation 4.5 cal; Institute/other 0.6

Name: Zhiquan Xiang, MD, Senior Staff Scientist
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 4.8

Contribution to Project: Dr. Xiang initially trained Dr. Areski, he is in charge of production and quality of vaccines and he assists Dr. Areski in handling of mice
Funding Support: Current proposal 4.8 cal; Virion 2.9 cal; Mathers Foundation 2.9 cal; Institute/other 1.4 cal

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

A Core Facility of the University of Pennsylvania provided melanoma samples and was reimbursed.

8. SPECIAL REPORTING REQUIREMENTS

N/A

9. APPENDICES:

N/A