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CONTRACTING ORGANIZATION: University of California, Los Angeles

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14. ABSTRACT

The object of this research is to develop a therapeutic strategy against lung cancer by utilizing novel vault bioparticles to deliver CCL21, an immunotherapeutic chemokine. Our hypothesis is that delivering CCL21 to the tumor, will make the entire repertoire of tumor antigens available in situ and thus increase the likelihood of an immune response and reduce the potential of phenotypic modulation. We have developed a bioparticle to deliver CCL21 that is based on the human vault particle. Vaults needed for initial animal studies (CCL21-vaults and empty control vaults) have been purified. We have secured ACURO approval for our animal studies and initial anti-tumor efficacy studies have been initiated in three animal models of lung cancer with the CCL21-vault as monotherapy. One model showed significant efficacy.

15. SUBJECT TERMS
Vault nanoparticles, CCL21 chemokine, Major Vault Protein (MVP), Non-small-cell lung carcinoma (NSCLC), Programmed cell death protein 1 (PD1).

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Introduction:

The object of this research is to develop a therapeutic strategy against lung cancer by utilizing novel vault bioparticles to deliver CCL21, an immunotherapeutic chemokine. Our hypothesis is that delivering CCL21 to the tumor, will make the entire repertoire of tumor antigens available in situ and thus increase the likelihood of an immune response and reduce the potential of phenotypic modulation. We have developed a bioparticle to deliver CCL21 that is based on the human vault particle. Vaults are nano-scale particles consisting of an outer protein shell comprised of 78 copies of a single protein called the major vault protein (MVP). We are able to manufacture empty vault shells that have remarkable homogeneity and stability and we have taken advantage of a high-affinity binding domain (termed INT) to package the human CCL21 chemokine into these human vault shells. Using a mouse lung tumor syngeneic model, we showed that intratumoral (IT) injection of CCL21-vaults could promote the recruitment of T lymphocytes and antigen presenting cells into the tumor environment leading to a robust antitumor response. The Specific Aims of this proposal ware directed at confirming this approach and gathering pre-clinical data in three animal models of lung cancer with the CCL21-vault as monotherapy and in combination with a checkpoint inhibitor. We will also carry out preliminary toxicology studies in mice. This proposal will develop a therapeutic strategy against lung cancer and has the potential to be an effective therapy for other solid tumors.

Keywords:

PD-1 – Programmed cell death protein 1, also known as PD1 and CD279 (cluster of differentiation 279), is a cell surface receptor that plays an important role in down-regulating the immune system and promoting self tolerance by suppressing T cell inflammatory activity.

PD-L1 - Programmed death-ligand 1 (PD-L1) also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1) is a protein that in humans is encoded by the CD274 gene. PDL1 is the ligand for PD1. Both PD1 and PDL1 are immune regulatory checkpoint markers.

CD8+ T cells - A cytotoxic T cell that is a T lymphocyte that kills cancer cells, cells that are infected (particularly with viruses), or cells that are damaged in other ways. Also called cytolytic T cells or CTL.

CD4+ T cells – A type of T helper cell (Th cell) that play an important role in the immune system, particularly in the adaptive immune system. These cells express the surface protein CD4 and are referred to as CD4+ T cells.

TIL – Tumor infiltrating leukocytes are white blood cells that have left the bloodstream and migrated into a tumor. They are mononuclear immune cells, a mix of different types of cells (i.e., T cells, B cells, NK cells, macrophages) in variable proportions, T cells being the most abundant cells.

Treg – Regulatory T cells, formerly known as suppressor T cells, are a subpopulation of T cells which modulate the immune system, maintain tolerance to self-antigens, and prevent autoimmune disease.

MDSC - Myeloid derived suppressor cells are a heterogenous group of immune cells from the myeloid lineage.

NSCLC - Non-small-cell lung carcinoma (NSCLC) is any type of epithelial lung cancer other than small cell lung carcinoma (SCLC). NSCLC accounts for about 85% of all lung cancers.

MVP – Major Vault Protein (MVP) is the structural protein of the vault bioparticle. There are 78 copies of MVP in a vault shell.

INT – The Major Vault Protein Interaction Domain (INT) – is a protein domain derived from the vault protein VPARP that binds with nanomolar affinity to the inside of the vault particle.

CCL21 - A multifunctional chemokine that mediates migration of lymphocytes and antigen-stimulated DCs into T cell zones of secondary lymphoid organs.

CCL21-vault – A vault particle packaged with the CCL21 chemokine fused to the INT packaging domain (CCL21-INT).

Overall Project Summary:

The object of this proposal is to develop a therapeutic strategy against lung cancer by utilizing novel vault bioparticles to deliver an immunotherapeutic chemokine. CCL21 is a multifunctional chemokine that mediates leukocyte migration and activation. CCL21 recruits lymphocytes and antigen-stimulated DCs into T cell zones of secondary lymphoid organs, co-localizing these early immune response constituents and culminating in cognate T cell activation to recruit and activate T cells. Our hypothesis is that delivering CCL21 to the tumor, will make the entire repertoire of antigens available in situ and thus increase the likelihood of an immune response and reduce the potential of phenotypic modulation. We have developed a bioparticle to deliver CCL21 that is based on the human vault particle. Endogenous vault particles are found in great abundance in the cytoplasm of all nucleated human cells. Vaults are nano-scale particles consisting of an outer protein shell comprised of 78 copies of a single protein called the major vault protein (MVP). In human cells this protein shell protects two other vault proteins and a small RNA, however, by expressing MVP alone in insect cells (which lack endogenous vaults), empty vault shells are produced that are morphologically indistinguishable from endogenous human vaults, but devoid of normal internal contents. These recombinant human vault shells are remarkably stable and homogenous. We have taken advantage of a high-affinity binding domain (termed INT) to package the human CCL21 chemokine into these human vault shells. Using a mouse lung tumor syngeneic model, we showed that intratumoral (IT) injection of CCL21-vaults could promote the recruitment of T lymphocytes and antigen presenting cells into the tumor environment leading to a robust antitumor response. Our data also suggests that subcutaneous delivery of the CCL21-vault as a vaccine may be more effective than the IT route.

The Specific Aims of this proposal are directed at confirming this approach and gathering pre-clinical data in three animal models of lung cancer with the CCL21-vault as monotherapy and in combination with a checkpoint inhibitor. We initially also planned to carry out preliminary toxicology studies in mice, however, due to the significant delay and impact of the COVID pandemic, this aim was dropped from the scope of the grant. This revisal of the aims was approved when our second NCE was approved. This proposal will develop a therapeutic strategy against lung cancer and has the potential to be an effective therapy for other solid tumors. If successful, this work will have a clear path to human testing since human vaults have been produced at commercial scale and GMP purity through an NCI SBIR.

Key Research Accomplishments:

What were the major goals of the project?

Specific Aim 1: Evaluate the efficacy of human CCL21-human vault therapy in three murine models of lung cancer (months 1-12)

Major Tasks: 1. Prepare Vaults for Aim 1 Animal Testing

Milestone(s) Adequate vaults purified and characterized for carrying out Tasks 2 and 3. (100% complete)

2. Quantify efficacy of human CCL21-vaults in 3 murine models of lung cancer & optimize dose and route of CCL21-vault delivery

Milestone: ACURO Approval (100% complete)
Milestone(s): Efficacy determined in 3 mouse models, optimal dose and route determined. (60% complete)

3. Evaluate the determinants of anti-tumor response

Milestone: Determinants of anti-tumor response analyzed. (25% complete)

Specific Aim 2: Assess the efficacy of combination therapy with anti-PD-1 and human CCL21-vaults in murine models of lung cancer (months 10-24)

Major Tasks: 1. Prepare Vaults for Aim 2 Animal Testing

Milestone: Adequate vaults purified and characterized for carrying out Tasks 5 and 6. (100% complete)

2. Assess the efficacy of combination therapy with anti-PD-1 and human CCL21-vaults in murine models of lung cancer.

Milestone: Efficacy determined in 3 mouse models, optimal dose and route determined. (10% complete)

3. Evaluate the determinants of anti-tumor response

Milestone: Determinants of anti-tumor response analyzed. (0% complete)

Specific Aim 3: Assess safety of CCL21-vaults following systemic administration in mice (months 12-24) – This aim was dropped due to cost and time constraints (this revision was approved when our second NCE was approved).

What was accomplished under these goals?

As all non-COVID-19 related research in California and most of the US was severely curtailed from March 2020 untill June 2021, we were significantly delayed in accomplishing our goals. In addition, we applied for and were granted two no cost time extensions. However, as we will explain in this report, there is reason for optimism as we have now ramped up our research. The details of this progress report will rely heavily on our years one and two reports to clarify how we are dealing with our delay and moving forward. Thus this progress report is a cumulative report for years 1-3. We plan to issue a final progress report shortly after the NCE ends in October.

Specific Aim 1, Major Task 1: Over the first half year of our research on this grant we prepared vaults for the aim 1 animal testing experiments. The vaults for these studies were produced at GLP purity. We utilized the baculovirus protein expression system that we had previously developed to produce the human CCL21-human vault and the control, empty human vault. The quality and quantity of vaults produced using the baculovirus system were evaluated and confirmed to be of high purity and adequate yield using standard operating procedures developed in our lab. Although we had used the baculovirus expression system (which uses sf9 insect cells)

for many years in the research setting, we were limited by the system. While a good research tool, the baculovirus approach is complex and costly for industrial scale applications and therefore partnering with a company with on-going baculovirus expression was necessary for development of vaults using this expression system. The system also has stability problems, which is another reason it has found limited utility in the commercial space. Several years ago our laboratory formed a partnership with a vaccine company, Protein Sciences Corp. (PSC), one of the only commercially and FDA approved baculoviral manufacturers. With PSC we developed a GLP purification of sf9-expressed vaults funded by phase I and II SBIR grants from the National Cancer Institute. These grants allowed us to develop a GLP vault expression and purification process. Unfortunately as we were in the process of completing the GLP process and product stability studies, PSC was purchased by Sanofi and we were informed that Sanofi was not interested in producing our GMP vaults.

Fortunately for us we had been working in parallel on expression of vaults in yeast (*Pichia pastoris*). Like insects, yeast also lack native vaults and, more importantly, yeast are a much more robust commercial manufacturing system. In addition to demonstrating that the yeast-expressed human vaults were structurally indistinguishable from sf9-expressed vaults, we also showed that the commercial-scale GLP purification procedures that we had developed were also transferrable to yeast expressed vaults. Because of the above reasons, we pivoted our laboratory vault production system to yeast. Over the past three years we have now entirely switched our vault manufacturing process to yeast. This change is also discussed below under the **Changes/Problems** section of this report.

Specific Aim 1, Major Task 2: As discussed above, due to a delay in securing our ACURO approval, we initiated these experiments in January 2019. To date we have evaluated the efficacy of human CCL21-vaults in 3 novel murine models of lung cancer with increased mutational loads.

Traditional conditional genetically-engineered murine models (GEMMs) of NSCLC bear driver mutations of the disease, recent studies reveal these GEMMs possess low mutational burden, and have shown limited utility in preclinical studies of immunotherapy. We have established novel GEMMs that better recapitulate the mutational landscape of human NSCLC by bearing common driver mutations and varying mutational loads. Previously propagated cell lines with known driver mutations ($kras^{G12D}$ (LKR-13); $kras^{G12D}/p53^{-/}$ (KP); and $kras^{G12D}/p53^{-/-}$, $lkb1^{-/-}$(KPL)) were exposed to MNU treatment three times to increase the mutational load. These cell-lines are labeled as LKR-13-3M, KP-3M, and KPL-3M.

We evaluated the efficacy of the CC21-vault in these three-cell line. CCL21-vault monotherapy did not show efficacy in the KP-3M and KPL-3M mouse models. However, treatment of LKR13-3M with human CCL21-vaults as a single arm trial revealed significant anti-tumor responses (Fig 1).

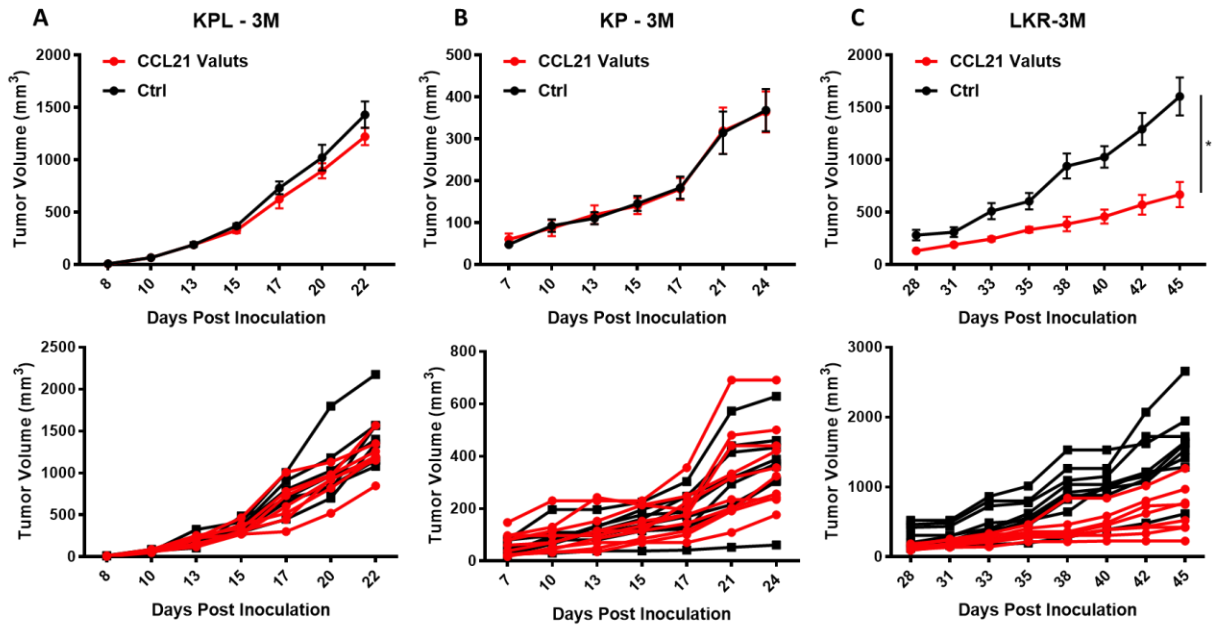

Figure 1. A) Post-tumor inoculation (1x10^5 KPL-3M cells delivered SC), FVB mice bearing <50mm^3 tumors (~d5-7) were treated with i) vehicle, ii) Human CCL21-vault (50µg/dose IT every three days for 3 doses), and tumor growth was measured with caliper. **B)** Post-tumor inoculation (1x10^6 KP-3M cells delivered SC), FVB mice bearing <50mm^3 tumors (~d5-7) were treated with i) vehicle, ii) Human CCL21-vault (50µg/dose IT every three days for 3 doses), and tumor growth was measured with caliper. **C)** Post-tumor inoculation (2x10^6 LKR-13-3M cells delivered SC), 129/E mice bearing <50mm^3 tumors (~d5-7) were treated with i) vehicle, ii) Human CCL21-vault (50µg/dose IT one times per week for 4 doses), and tumor growth was measured with caliper. *P* values were determined by non-paired *t*-test. *, *P*<0.05; **, *P*< 0.005; ***, *P*<0.0005; ****, *P*< 0.0001.

Conclusions:

We have made solid progress on the Aims of year one and we are on track for successfully completing all of our aims except Aim 3 by the end of the no cost time extension period.

What opportunities for training and professional development has the project provided?
Nothing to Report.

How were the results disseminated to communities of interest?
Nothing to Report.

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

During the next reporting period we plan to accelerate the testing of CCL21-vaults in animal models & optimize the dose CCL21-vault delivery (Specific Aim 1). We will also evaluate the determinants of anti-tumor response (Specific Aim 1). We will confirm efficacy of the CCL21-vault in the LKR-3M1 and 3LL tumor models (this later model was previously shown to be suppressed by CCL21-vaults) and continue to focus on Specific Aim 2 where we will assess the efficacy of combination therapy with anti-PD-1 and human CCL21-vaults in these two murine models of lung cancer.

For the experiments described above, we plan to prepare the vaults using the yeast protein expression system.

Impact: Nothing to Report.

Changes/Problems: Of course the COVID-19 Pandemic has caused a significant delay in our 2020 research, although there are no significant changes in the project or its direction. We did change our vault manufacturing system from the baculovirus system to yeast. The reason for this change is described here.

We have used the baculovirus expression system (which uses sf9 insect cells) for many years in the research setting. While a good research tool, the baculovirus approach is complex and costly for industrial scale applications and therefore required that we partner with a company with on-going baculovirus expression for development of vaults using this expression system. The system also has stability problem which is another reason it has found limited utility in the commercial space. We formed a partnership with a vaccine company, Protein Sciences Corp. (PSC), one of the only commercially and FDA approved baculoviral manufacturers. With PSC we developed a GLP purification of sf9-expressed vaults funded by phase I and II SBIR grants from the National Cancer Institute. Unfortunately as we were finishing our GLP process and product stability studies, PSC was purchased by Sanofi and we were informed that Sanofi was not interested in producing our GMP vaults.

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Due to the pandemic, UCLA research was closed from March-June 2020, however, some COVID-19 related research was approved and we formed a group called the COVID-Vault Vaccine Multi-Institutional Research Initiative (COVID-Vault MIRI). The group included the following members: UCLA (Jeff F. Miller, PhD, (CNSI) and Leonard Rome, PhD (Biol Chem)), Northern Arizona University (F. Todd French, PhD (Pathogen and Microbiome Institute), Vault Pharma Inc. and the University of Nebraska, Lincoln (Biological Process Development Facility).

Although the object of COVID initiative was to create, develop and manufacture a candidate vault vaccine that aims to induce both an antibody and a robust T-cell response and thus impart protective immunity on recipients, ***I want to stress that some important overlaps exist between the aims of this initiative and the aims of our LCRP***. The COVID project had very limited funding, however, at UCLA we are producing candidate non-infectious SARS-CoV2 antigens, and antigens from related coronaviruses, fused to the vault packaging domain, using yeast protein expression protocols that we have been developing under the LCRP proposal in my laboratory. Thus some of the necessary steps were also necessary for our LCRP grant and our lead researcher, Syeda Khadija, Ph.D., was able to learn the yeast vault expression system and contribute to both projects. We were also able to enlist the Life Sciences Fermenter Core Facility at UCLA (Mark Arbing, PhD, Director) to carry out small-batch prototype yeast fermentations. Experiments that are critical to our LCRP grant's progress.

Experiments that we carried out under Aim 1 used baculovirus produced vaults prepared during year 1 of the grant. As we are now completely switched over to yeast manufacturing, all the vaults needed for Aim 2 studies are being produced in yeast with identical purity. We have initiated animal experiments to demonstrate the efficacy of the yeast produced vaults in the mouse 3LL tumor model. These changes will have no significant impact on expenditures.

Products: Nothing to Report.

Participants & Other Collaborating Organizations
What individuals have worked on the project?

Name:	*Leonard H. Rome*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-1236-2063*
Nearest person month worked:	*1.2*
Contribution to Project:	*Dr. Rome is overseeing the entire project and participating in assessment of vault purity.*
Funding Support:	

Name:	Syeda Khadija
Project Role:	*Staff Research Associate*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*7.84*
Contribution to Project:	*Dr. Khadija is responsible for purifying vault particles for use in the animal experiments.*
Funding Support:	

Name:	Francisco Maciel
Project Role:	*Undergraduate Student Researcher*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*0.6*
Contribution to Project:	*Prepared buffers, washed glassware etc.*
Funding Support:	

Name:	*Ramin Salehi-rad*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1.2*
Contribution to Project:	*Dr. Salehi-rad is in charge of all of the animal experiments.*
Funding Support:	

Name:	*Bin Liu*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*2.4*

Contribution to Project:	*Dr. Liu is in charge of determining the determinants of response to the CCL21-vaults. In addition she is assisting Dr. Salehi-Rad with the animal experiments.*
Funding Support:	

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

There has been one change in key personnel since the grant was funded. Stacy Park, Ph.D., Co-Investigator has taken another position and has been replaced by Dr. Bin Liu. Dr. Liu is an Adjunct Professor in the Department of Medicine with expertise in immunology, molecular and cell biology. She will oversee the flow cytometry analysis in order to determine the determinants of response to the CCL21-vaults. In addition she has been assisting Dr. Salehi-Rad with the animal experiments.

There has also been a change in the "Other Personnel" category. Dr. Rome's technician, Hedi Roseboro, retired from UCLA and was originally replaced by Ms. Joye Yang who left in September 2019 and was replaced by Dr. Syeda Khadija, Staff Research Associate. As mentioned above, Dr. Khadija was trained to take over Ms. Yang's responsibilities in the lab and she has been providing assistance with all aspects of the project that require production and purification of CCL21-vaults and empty vaults. She is now fully trained to produce vaults in yeast and she is in charge of day-to-day particle production and purification for the experiments described in Aims 1-2.

What other organizations were involved as partners?
Nothing to Report