**Project Information**

**Project Title**

B7-H3 specific CAR T cell based combinatorial immunotherapy for intrahepatic cholangiocarcinoma

**Amount Requested**

100,000

** Applicant Information **

**Applicant Name**

Theodoros Michelakos

**eRA Commons Username**

TPMICHELAKOS

**Degree (Select all that apply)**

- MD

**Academic Level (Select all that apply)**

- Post-Doc Fellow

**Institution Information**

**Institution Name**

Massachusetts General Hospital

**Institution City**

Boston

**Institution State**

Massachusetts

**Institution Country**

United States

**Email**

tmichelakos@partners.org

**Mentor Information**

**Mentor Name**
2017 Research Fellowship Application : Entry #11029

Cristina R. Ferrone

Mentor Title

Associate Professor

Mentor Institution

Massachusetts General Hospital

Submission Information

Scientific Abstract (1500 Max Characters)

The need for a therapy for ICC prompted us to develop a safe and effective combinatorial chimeric antigen receptor (CAR) T cell-based immunotherapy, by taking advantage of the mentors' expertise in clinical aspects of ICC (C.R. Ferrone), immune escape mechanisms (S. Ferrone) and CAR T cell generation (G. Dotti). This multi-disciplinary approach is expected to train the applicants in areas crucial for an effective treatment of ICC.

T cells transduced with the B7-H3 CAR we generated can eradicate ICC cells in vitro. The two trainees will test whether the B7-H3 CAR T cells can eradicate ICC cells cultured in vitro under hypoxia, or grafted in immunodeficient mice. This project will be an excellent training vehicle since the trainees will be exposed to a variety of methods and more importantly will be involved in the design of experiments and in the interpretation of results. This arrangement will provide the two trainees with a robust background to establish independent ICC focused research programs.

Since the limited success of CAR T cells in solid tumors may be caused by hypoxia-induced escape mechanisms, CAR T cells will be combined with strategies which counteract them. Specifically, an anti-PD-1 scFv was incorporated in the CAR construct to inhibit the PD-L1/PD-1 axis which "exhausts" CAR T cells. Additionally, sonidegib, which inhibits the activation of the Sonic Hedgehog Homologue pathway and the subsequent up-regulation of anti-apoptotic molecules, is administered.

Research Summary (1500 Max Characters)

Due to the lack of therapies for ICC, we developed a novel combinatorial immunotherapy. We use T cells transduced with a B7-H3-specific chimeric antigen receptor (CAR) as effectors, since this approach provides both tumor specificity and self-amplification. The mAb 376.96-defined B7-H3 epitope was selected as a target, since it is expressed selectively on tumor cells and tumor vasculature, but has a more restricted distribution in normal tissues, than the B7-H3 molecule. Furthermore, it is expressed on cancer initiating cells, which need to be eradicated for a therapy to be effective, because of their role in recurrence and metastasis.

The postulated role of the tumor microenvironment hypoxia in the limited efficacy of CAR T cell therapies in solid tumors has prompted us to develop strategies to counteract the hypoxia-induced escape mechanisms utilized by ICC cells to avoid immune destruction. To this end, we have incorporated an anti-PD-L1 scFv in the CAR construct to block the PD-L1/PD-1 axis which "exhausts" T cells. Furthermore, we combine CAR T cells with sonidegib, to inhibit the Sonic Hedgehog Homologue (SHH) pathway activation, which leads to anti-apoptotic molecule up-regulation. The validity of our approach will be tested in vitro under hypoxic conditions, and in an orthotopic mouse model.

Since we plan to translate our strategy to a clinical setting, we have incorporated a highly effective safety switch in the CAR construct, to ensure the safety of our approach.

Lay Summary (1500 Max Characters)
The goal of this proposal is to provide two post-doctoral fellows with a robust background to establish independent ICC focused research programs. To this end, they will be supervised by three independent investigators experienced in distinct but complementary research areas relevant to the proposed study. The research program used as a training vehicle aims to implement a safe and effective combinatorial immunotherapy for the treatment of ICC. T lymphocytes genetically engineered to specifically recognize ICC cells and kill them are used as effectors. The target of the generated T cells is a molecule selectively expressed on ICC cells. Additionally, it is expressed on a sub-population of ICC cells, which play a major role in recurrence and metastasis. Therefore, our strategy is expected to be both effective and safe.

We have shown that genetically engineered T cells can kill ICC cells in vitro. To enhance the anti-tumor activity of the T cells, they are combined with strategies which counteract the resistance mechanisms caused by hypoxia, a hallmark of tumor microenvironment. Specifically, we minimize the inhibitory signals released by tumor cells to T cells and we restore the susceptibility of ICC cells to T cell killing.

The validity of the described strategy will be tested with human ICC cells grafted in mice. The results obtained from this study will provide a useful background to move the described strategy a clinical setting.

Specific Aims (2500 Max Characters)

The lack of effective therapies for ICC prompted us to develop a novel combinatorial immunotherapy. T cells transduced with a B7-H3-specific chimeric antigen receptor (B7-H3 CAR T cells) are used as effectors, since this approach allows specificity of tumor recognition and self-amplification due to self-renewal capacity of T lymphocytes.

The B7-H3 epitope recognized by our monoclonal antibody (mAb) 376.96 used to generate the CAR is expressed on the 3 ICC cell lines and on the 5 surgically removed ICC tumors tested (Figs. 1,2). Noteworthy, B7-H3 is expressed on both ICC differentiated cells and ICC cancer initiating cells (CICs). CICs have to be eradicated for a therapy to be successful, since they play a major role in recurrence and metastasis(1, 2). The B7-H3 epitope is also expressed in the tumor-associated vasculature and fibroblasts(3). Therefore, B7-H3 immunotargeting is expected to inhibit neoangiogenesis, contributing to the elimination of ICC cells, even those with low or non-detectable B7-H3 expression. The mAb 376.96-defined B7-H3 epitope is not detectable in other normal tissues, thus minimizing the side effects caused by B7-H3 targeting.

We have shown that B7-H3 CAR T cells can eradicate ICC differentiated cells and ICC CICs cells in vitro in normoxia (Fig. 5). CAR T cells are less effective with solid tumors in vivo because of hypoxia induced escape-mechanisms. The trainees will therefore test the hypothesis that the in vivo anti-tumor activity of CAR T cells can be markedly enhanced by disrupting the PD-1/PD-L1 axis and by inhibiting the SHH pathway, for the reasons indicated in the Background section. Therefore they will test that:

1. The anti-tumor activity of B7-H3 CAR T cells incubated in vitro with ICC cells under hypoxia is enhanced by anti-PD-L1 scFv and by sonidegib.
2. The in vivo relevance of the in vitro results will be tested by assessing the ability of B7-H3 CAR T cells secreting anti-PD-L1 scFv in combination with sonidegib to eradicate ICC cells orthotopically grafted in mice, and to prolong their survival.

The trainees have participated in the generation of the CAR, have generated the preliminary results and have designed the research proposal with the mentors’ input. They plan to communicate on a bi-weekly basis by videoconference with the mentors to review the results and plan future experiments. They will also draft the manuscripts and discuss them with the mentors. They will be encouraged to attend relevant seminars.

Background and Significance (3000 Max Characters)
The lack of effective therapy for ICC prompted us to develop a safe and novel combinatorial CAR T cell-based immunotherapy. Our strategy is expected to be highly effective, since i) it targets both differentiated and cancer initiating cells, ii) it counteracts the hypoxia-induced escape mechanisms utilized by ICC cells to avoid immune destruction, and iii) because of its HLA-independence averts the negative impact of HLA class I antigen defects. The latter are frequently present in ICC tumors (4).

B7-H3, a B7 family member, has been selected as a target, since the epitope recognized by our mAb 376.96 is highly expressed on both ICC cell lines and tumors, but has a restricted distribution in normal tissues. This epitope is not detectable in normal tissues which express the B7-H3 molecule. Therefore, we anticipate that B7-H3 immunotargeting is not likely to cause side effects mediated by targeting normal cells.

We have generated the B7-H3 CAR as described in Fig. 3. T cells transduced with this CAR are effective in completely eradicating ICC cells in vitro under normoxic conditions (Fig. 5). However, the anti-tumor activity of CAR T cells with solid tumors in vivo is limited. Our studies with other types of solid tumors indicate that this reduced activity is caused by hypoxia-induced changes. They include i) PD-L1 induction on tumor and immune cells. The resulting interaction of PD-L1 with PD-1 expressed by T cells inhibits their anti-tumor activity; and ii) activation of the SHH pathway, which in turn upregulates anti-apoptotic molecules. To disrupt the PD-1/PD-L1 axis we incorporate an anti-PD-L1 scFv in the CAR (B7-H3 CAR anti-PD-L1 scFv T cells) (Fig. 4). Our strategy has the advantage to act locally and minimize the side effects caused by the systemic administration of anti-PD-1 or anti-PD-L1 antibodies. To restore ICC cell sensitivity to T cell-mediated lysis we administer sonidegib, an FDA approved SHH pathway inhibitor.

We are aware that infusion of CAR T cells can cause toxicity related to the induction of the cytokine release syndrome. For safety purposes, given our expectation to translate our findings to a clinical setting, we have prepared a B7-H3 CAR construct incorporating a highly effective safety switch based on the inducible caspase9 transgene (iC9) (iC9-CAR)(ref). Activation of the iC9 safety switch mediated by the specific chemical inducer of dimerization will allow the selective elimination of CAR T cells.

**Project Timeline (2500 Max Characters)**

The duration of Specific Aims 1 and 2 will be 3 and 9 months, respectively.

**Aim 1.** The anti-tumor activity of B7-H3 CAR T cells incubated in vitro with ICC cells under hypoxia is enhanced by anti-PD-L1 scFv and by sonidegib. Months 1-3.

1.1. ICC cell growth inhibition by our combinatorial immunotherapeutic strategy. The luciferease transfected cell lines ICC2-Luc and ICC3-Luc will be used. The optimal dose of sonidegib and incubation time will be determined. ICC cells will be treated with sonidegib in 1% hypoxia. Then cells will be co-cultured with CAR T cells (E:T=5:1) for 7 days. Residual tumor cells and T cells will be assessed by flow cytometry. The IFNγ release assay will also be performed.

1.2. ICC CICs growth inhibition by our combinatorial immunotherapeutic strategy. After repeating experiments described in 1.1, the percentage of ALDH(bright) & CD133(+) cells will be determined before and after treatment.

1.3. Statistics. We will use one-way ANOVA test.

1.4. Alternative strategies. If the sonidegib protocol is not effective, we will adjust its dose and incubation time. If the targeted B7-H3 epitope is lost, we will use a CAR we have constructed with the Grp94-specific mAb W9. If the anti-PD-L1 scFv is not effective, we will use an anti-PD-1 mAb.

**Aim 2.** The in vivo relevance of the in vitro results will be tested by assessing the ability of B7-H3 CAR T cells secreting anti-PD-L1 scFv in combination with sonidegib to eradicate ICC cells orthotopically grafted in mice, and to prolong their survival. Months 4-12.

2.1. B7-H3 CAR T cells are effective in suppressing tumors in NSG mice. ICC2-Luc cells will be orthotopically grafted in mice. Tumor size will be determined by bioluminescent imaging. Mice will be separated in 6 treatment groups (Table 1). Tumor size and survival will be recorded. Results will be validated with the ICC3-Luc cell line.

2.2. Characterization of tumors. CIC percentage, targeted epitope loss, anti-apoptotic molecule expression, and anti-angiogenic effects will be analyzed.

2.3. Side effects. Mouse health will be monitored.

2.4. Statistics. We will use one-way ANOVA, Kaplan-Meier and log-rank tests.

2.5. Alternative strategies. If the described strategy does not eradicate tumor cells and prolong survival, we will replace the anti-PD-L1 scFv with an anti-PD-1 mAb. If our strategy is still not effective we will combine it with gemcitabine. If the study is underpowered we will increase the sample size accordingly.

**Literature Cited**
2017 Research Fellowship Application: Entry # 11029


Supporting Files - Please upload any supporting files such as charts or graphs in JPG or PDF format.

* 1234.pdf

Budget

Impact or Collab Award

I want to apply for a Collaboration Award (up to $100,000; requires multi-institutional collaboration)

Collab Institution 1

Massachusetts General Hospital

Collab Applicant 1

Theodoros Michelakos

Collab App 1 Title

Research Fellow

Collab App 1 Effort

100

Collab App 1 Base Salary

42800

Collab App 1 Salary Requested

42800

Collab Institution 2

University of North Carolina at Chapel Hill, School of Medicine
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**Collab App 2 Title**

Postdoc

**Collab App 2 Effort**

100

**Collab App 2 Base Salary**

44840

**Collab App 2 Salary Requested**

44840

**Collab App 2 Salary Requested**

87640

**Collab Equipment List**

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**Collab Total Requested**

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