

CSF as the main candidate mediators of this skewing of monocytes to an M2-like state. The use of specific neutralizing antibodies against each of these cytokines prevented the observed DC suppression to varying degrees. t-Distributed Stochastic Neighbor Embedding (t-SNE) identified specific shifts between monocytic subpopulations and modulated expression levels of associated surface markers. Neutralization of M-CSF reduced expression of BDCA3, PD-L2, and PD-L1, while increased CD16; whereas blocking TGF-beta led to a concerted reduction in CD14, CD163, PD-L1, and PD-L2 levels, but, unexpectedly, also of CD80. In contrast, IL-10 neutralization resulted in a decrease of all M2-related markers, while CD80 levels were upregulated. Interestingly, while the SK-MEL-28 cell line did not secrete detectable levels of IL-10 in traditional monolayer cultures, RNA *in situ* hybridization revealed *de novo* expression in Mel-RhS in melanoma cells, as well as in keratinocytes and fibroblasts.

**Conclusions** We conclude that the 3D configuration of the Mel-RhS model results in cross-talk between tumor and stroma, which allows for the delineation of immune suppressive pathways in the melanoma TME. Ultimately, this model could be used as a novel *in vitro* tool for preclinical testing of immune modulatory therapeutic agents.

**Disclosure Information** M. Lopez Gonzalez: None. E. Michielon: None. J.L.A. Burm: None. T. Waaijman: None. E.S. Jordanova: None. S. Gibbs: None. T.D. de Gruijl: None.

## On Demand Talks: Combination Therapy

06

### EXPRESSION OF ANTI-APOPTOTIC GENE CFLIP TO ENHANCE PERSISTENCE IN CAR T CELLS

GMY Tan\*, SMAR Hosseini, A Poudel, AD Mcllellan. *University of Otago, North Dunedin, New Zealand*

10.1136/jitc-2020-ITOC7.11

**Background** CAR T cell therapy has been successful for targeting blood cancers, but treatment of solid cancers has been limited due to the heterogenous nature of tumour-associated antigen expression on solid cancers, and the suppressive tumour microenvironment.<sup>1</sup> Another major obstacle to CAR T cell therapy is activation-induced cell death (AICD) of the CAR T cells.<sup>2</sup> In this study, we expressed the anti-apoptotic cellular FLICE-like inhibitory protein (c-FLIP short; c-FLIPs) together with the CAR construct to enhance CAR T cell persistence.<sup>3</sup>

**Materials and Methods** The anti-Her2 FRP5 CAR T construct with P2A-linked cFLIPs or cFLIPp43 was cloned into the Sleeping Beauty (SB) transposon vector (pSBtet-GP) or lentiviral vector, under the control of either a tet-on or a constitutive promoter. Construct expression was validated by qPCR and immunoblot analysis. CAR T cells were generated by SB transposition or lentiviral transduction of CD3/CD28 stimulated primary human T cells that were subsequently maintained with IL-2. Mitochondrial function and apoptosis were determined by resazurin assay and by flow cytometry using tetramethyl rhodamine (TMRE).

**Results** Overexpression of cFLIP (cFLIPp43 and cFLIPs) in pSBtet-GP demonstrated protection in both Jurkat T cell line and primary human T cells. pSBtet-GP was modified to

overexpress cFLIPs and cFLIPp43 under tet-on promoter, with the anti-her2 CAR, GFP and rtTA under constitutive promoter. Transfer of the inducible cassette from the SB transposon to a lentiviral system resulted in a significant loss of tightness. Doxycycline treated CAR T cells showed only ~13-fold overexpression of cFLIPs or cFLIPp43 compared to untreated cells, and doxycycline significantly inhibited (approximately 30%) primary CAR T cell expansion. In contrast, constitutive expression of CAR-cFLIPs or cFLIPp43 construct gave a  $>3 \times 10^5$ -fold cFLIP overexpression, as compared to CAR-only control. While the transduction efficiency of CAR-only was around 70–80% control in primary T cells, this dropped to 20–25% when using the more genetically complex tet-on system.

**Conclusions** cFLIP protects T cells from Fas-induced apoptosis. The tet-on system demonstrates several drawbacks in the lentiviral system, including toxicity of the inducer drug (and/or squelching effects resulting in lowered viability), loss of responsiveness and lowered transduction frequencies. Therefore, a constitutive promoter system is preferred in lentiviral systems for the control of genes of interest within CAR T cells, while the SB transposon system may be preferred for tet-on control within CAR T cells.

### REFERENCES

1. Pitt JM, Marabelle A, Eggermont A, Soria JC, Kroemer G, and Zitvogel L. Targeting the tumor microenvironment: removing obstruction to anticancer immune responses and immunotherapy. *Ann Oncol* 2016;**27**:1482–1492.
2. Yeku O, Li X, Brentjens RJ. Adoptive T-Cell Therapy for Solid Tumors. *Am Soc Clin Oncol Educ Book* 2017;**37**:193–204.
3. Dohrman A, Kataoka T, Cuenin S, Russell JQ, Tschopp J, and Budd RC. Cellular FLIP (long form) regulates CD8+ T cell activation through caspase-8-dependent NF-kappa B activation. *J Immunol* 2005; **174**:5270–5278.

**Disclosure Information** G.M.Y. Tan: None. S.M.A.R. Hosseini: None. A. Poudel: None. A.D. Mcllellan: None.

07

### A BISPECIFIC VHH APPROACH TO LEVERAGE THE POTENT AND WIDELY APPLICABLE TUMOR CYTOLYTIC CAPACITY OF Vγ9Vδ2 T CELLS

<sup>1</sup>LA King\*, <sup>1</sup>R Lameris, <sup>2</sup>RC Roovers, <sup>2</sup>P Parren, <sup>1</sup>TD de Gruijl, <sup>1,2</sup>HJ van der Vliet. <sup>1</sup>AUMC – Cancer Centre Amsterdam, Amsterdam, Netherlands; <sup>2</sup>LAVA Therapeutics, Utrecht, Netherlands

10.1136/jitc-2020-ITOC7.12

Vγ9Vδ2-T cells include a unique and potent subset of T cells which play an important role in tumor defense. Vγ9Vδ2-T cells recognize and can lyse butyrophilin 3A1-expressing target cells with elevated levels of non-peptide phosphoantigens (pAg), induced by cell stress or malignancy. To date, Vγ9Vδ2-T cell based cancer immunotherapeutic approaches were well tolerated and in some cases capable of inducing relevant clinical responses. In an effort to improve the efficacy and consistency of Vγ9Vδ2-T cell based cancer immunotherapy, we designed a bispecific VHH that binds to both Vγ9Vδ2-T cells and EGFR expressed by tumor cells and results in the target-specific activation of Vγ9Vδ2-T cells and subsequent lysis of colorectal cancer cell lines and primary colorectal cancer samples both *in vitro* and in an *in vivo* mouse xenograft model. Of note, tumor cell lysis was independent of mutations in KRAS and BRAF that are known to impair the efficacy of clinically registered anti-EGFR monoclonal antibodies as well

as common V $\gamma$ 9V $\delta$ 2-T cell receptor sequence variations. In combination with the conserved monomorphic nature of the V $\gamma$ 9V $\delta$ 2-TCR and the facile replacement of the tumor-specific VHH, this immunotherapeutic approach can in principle be applied to a large group of cancer types.

**Disclosure Information** L.A. King: None. R. Lameris: None. R.C. Roovers: None. P. Parren: None. T.D. de Gruijl: None. H.J. van der Vliet: None.

### 08 GEMCITABINE INDUCES PRO-APOPTOTIC BH3 ONLY PROTEINS AND SENSITIZES PANCREATIC DUCTAL ADENOCARCINOMA CELLS FOR RLH-TRIGGERED IMMUNOGENIC CELL DEATH

<sup>1</sup>P Metzger\*, <sup>1,2</sup>HT Bourhis, <sup>1,3</sup>M Stieg, <sup>1,2</sup>D Böhmer, <sup>1</sup>S Endres, <sup>1,4</sup>P Düwell, <sup>1</sup>LM König, <sup>1</sup>M Schnurr. <sup>1</sup>Center of Integrated Protein Science Munich (CIPSM) and Division of Clinical Pharmacology, University Hospital, LMU Munich, Munich, Germany; <sup>2</sup>Department of Medicine II, University Hospital, LMU Munich, Munich, Germany; <sup>3</sup>Department of Medicine IV, University Hospital, LMU Munich, Munich, Germany; <sup>4</sup>Institute of Innate Immunity, University of Bonn, Bonn, Germany

10.1136/jitc-2020-ITOC7.13

**Background** Despite tremendous effort, the prognosis of patients with pancreatic ductal adenocarcinoma (PDAC) remains poor and therapy options are limited. Recent advances in chemotherapeutic schemes have increased the survival of PDAC patients by a few months only. So far, the success of immunotherapy seen in other cancer types could not be transferred to PDAC. Our group has demonstrated that single agent RIG-I-like helicase (RLH)-targeting immunotherapy induces an anti-tumoral immune response and improves survival in a PDAC mouse model dependent on the induction of immunogenic cell death. In addition, we and others were able to show that tumor cell death induction by RLH ligands is partially dependent on the induction of the pro-apoptotic BH3-only proteins PUMA and NOXA. In the current study we aim at improving therapy response using a combinatorial chemo-immunotherapy (CIT) approach.

**Methods** Tumor cell death induction by gemcitabine, oxaliplatin and 5-fluorouracil (5-FU) alone or in combination with RLH ligands was evaluated in the murine cell line Panc02. The induction of PUMA and NOXA was measured by real-time PCR. The capability of chemo-immunotherapy -induced tumor cell death to activate splenic CD8a<sup>+</sup>dendritic cells (DC) as well as to induce antigen uptake and cross-presentation was investigated *in vitro*. Therapeutic efficacy was evaluated *in vivo* using an orthotopic PDAC mouse model.

**Results** Gemcitabine, oxaliplatin and 5-FU induced dose-dependent tumor cell death *in vitro*. however, only gemcitabine lead to an induction of the pro-apoptotic proteins PUMA and NOXA. Simultaneous treatment with gemcitabine and RLH-ligand increased cell death induction without affecting the cytokine secretion substantially. CD8a<sup>+</sup> DC activation upon RLH-therapy was not affected by chemotherapy. Of note, antigen uptake as well as T cell priming was increased by chemo-immunotherapy. Most importantly, the survival of orthotopic PDAC bearing mice was significantly prolonged in the chemo-immunotherapy group compared to single agent treatment.

**Conclusions** Gemcitabine treatment of PDAC induces PUMA and NOXA expression which leads to mitochondrial priming and sensitization towards RLH-induced cell death. chemo-

immunotherapy increases the cross-presentation capability of DC *in vitro* and prolongs the survival of PDAC bearing mice. chemo-immunotherapy is therefore an attractive combinatorial therapeutic approach in PDAC.

**Funding** The project was supported by the Deutsche Forschungsgemeinschaft (DFG) - Projektnummer 179062510 and 329628492 - SFB 1321 as well as the Förderprogramm für Forschung und Lehre (FöFoLe) funded by the Ludwig-Maximilians-Universität München.

**Disclosure Information** P. Metzger: None. H.T. Bourhis: None. M. Stieg: None. D. Böhmer: None. S. Endres: None. P. Düwell: None. L.M. König: None. M. Schnurr: None.

## E-Poster Presentations

### P01 Emerging concepts/novel agents

#### P01.01 A PHASE 1A/1B DOSE-ESCALATION STUDY OF INTRAVENOUSLY ADMINISTERED SB 11285 ALONE AND IN COMBINATION WITH NIVOLUMAB IN PATIENTS WITH ADVANCED SOLID TUMORS

<sup>1</sup>A ABBAS\*, <sup>2</sup>J Strauss, <sup>3</sup>F Janku, <sup>4</sup>R Karim, <sup>5</sup>A Olszanski, <sup>6</sup>JJ Luke, <sup>1</sup>K Leach, <sup>1</sup>R Iyer. <sup>1</sup>Spring Bank Pharmaceuticals Inc, Hopkinton, MA, USA; <sup>2</sup>Mary Crowley Medical Research Center, Dallas, TX, USA; <sup>3</sup>MD Anderson Cancer Center, Houston, TX, USA; <sup>4</sup>Next Oncology, San Antonio, TX, USA; <sup>5</sup>Fox Chase Cancer Center, Philadelphia, PA, USA; <sup>6</sup>UPMC Hillman Cancer Center, Pittsburgh, PA, USA

10.1136/jitc-2020-ITOC7.14

**Background** Immunotherapy has emerged as a transformative approach for the treatment of cancer. However, a significant percentage of patients are nonresponsive to these immunotherapies or experience disease relapse which highlights the need for new therapies. Recent work has highlighted a major role for Stimulator of Interferon Genes (STING) agonists in immunotherapy. Conceptually, the activation of the STING pathway in immune cells in the tumor microenvironment (TME) and tumor cells could result in the induction of innate and adaptive immunity and subsequent activation of cytotoxic T cells and NK cells for durable anti-tumor responses. SB 11285 is a novel agonist of STING pathway leading to the activation of tumor-resident APCs and priming of tumor antigen specific CD8<sup>+</sup> T cells. In our preclinical studies using multiple tumor-derived cell lines, SB 11285 has been observed to cause the induction of cytokines, such as INF- $\beta$ , INF- $\alpha$ , TNF $\alpha$  and others consistent with engagement of the STING target, as well as tumor cell death by STING-mediated apoptosis. SB 11285 reduced tumor volumes in multiple rodent tumor models when administered intravenously, intraperitoneally and intratumorally. Systemic administration could additionally facilitate trafficking of newly activated CD8<sup>+</sup>T cells from periphery into the tumor site. In addition, preclinical models indicate that survival and local tumor shrinkage were significantly enhanced when SB11285 was administered with anti-CTLA-4 or anti-PD-1 antibody, suggesting that SB 11285 can be administered with anti-PD-1 and anti-CTLA-4 antibody for synergistic activity. A multiple ascending dose, phase 1a/1b trial of SB11285 in multiple tumor types has been initiated and the objectives of this trial include determining a safe and efficacious dose of intravenous SB 11285 and a preliminary assessment of antitumor activity/efficacy as either monotherapy or in combination with nivolumab.