Highlights from SITC 2021

Cell and Gene Therapy in Solid Tumors – Ray of Hope on the Horizon?

In a late-breaker at the SITC conference, Immatics presented impressive data for IMA203, an engineered TCR targeting PRAME antigen. In 16 evaluable subjects with highly refractory solid tumors, IMA203 demonstrated signs of efficacy in multiple tumors with ORR of ~50%. Promising data with TCR T-cell therapies has been witnessed lately in solid tumors. Adaptimmune and Immatics have demonstrated efficacy with their TCR T-cell therapies against respective cancer testis antigens MAGEA4 and PRAME. Early data for BioNTech's novel Claudin 6 targeted CAR-T platform and Iovance Therapeutics' adoptive cell therapy combination with anti-PD-1 pembrolizumab also reflected evolution of cellular therapies in solid tumors.

SITC 2021 included many novel approaches to improve the functionality of CAR-T and TCR magic bullets, and enhance their efficacy/safety by reprogramming the tumor microenvironment. Several approaches for the activation of stimulatory/co-stimulatory signals, disruption of negative immune regulators, and improvements in trafficking and homing were also covered. Innovations that involve engineering of not only T and NK cells but also diversity of other immune cell sources including induced pluripotent stem cells (iPSCs) and myeloid cells were also discussed.

SmartAnalyst is constantly monitoring the development in the cell and gene therapy (CGT) arena, and has selected some interesting SITC presentations to understand the challenges in development in solid tumors.

What are the developments in optimization of stimulation/co-stimulation to enhance CAR-T efficacy?

Cytokines play a crucial role in initiation and maintenance of T-cell-mediated immune responses, which are usually downregulated in TME. It was therefore natural to consider incorporation of cytokines in CAR constructs. Co-expression of immune modifying cytokines has been shown to improve cell viability, expansion, and potency. Three studies presented at SITC showed that cytokine armored CAR-Ts had better anti-tumor activity. ***Armored NKG2D CAR-T cells that included the IL-18 transgene showed prolonged sequential target cell killing as compared to non-armored CAR-Ts and increased levels of interferon gamma secretion upon antigen challenge*** *(Celyad Oncology).*[1] At high doses, some toxicity was seen in tumor bearing models which was abrogated by systemic infusion of human IL-18 binding protein (IL-18BP).

Autocrine and paracrine loops of ***Interleukin-2*** can improve CAR-T functionality in solid tumor and represent a promising strategy. Engineered IL2 which preferentially activates β/γ subunit overcome these challenges. A novel second generation mesothelin (***MSLN) specific CAR constructs*** (MSLN-CAR-T-IL-2tb) were designed that incorporate ***secretory form of IL-2 variants (IL-2tb) (GracellBio)***.[2] This IL-2 variant was able to maintain long-term proliferation and cytotoxicity which could be partly due to the reduced immunosuppression in the TME. IL-2tb produced by MSLN-CAR-T-IL-2tb improved cell viability, expansion, and potency, and reduced immunosuppression. MSLN-CAR-T-IL-2tb was active against multiple MSLN-expressing tumor cells and xenograft mouse models, demonstrating potent and durable anti-tumor responses.

What are the strategies to overcome CAR-T cell exhaustion?

CAR-Ts have been transformational in hematologic malignancies, however, very few patients respond for a prolonged period. Long-term responders are even rarer in solid tumors. One of the limitations against durable responses for CAR-Ts is their dysfunctional state during prolonged antigen exposure termed as ***CAR-T cell exhaustion***. The exhaustion of human CAR-T cells occurs through an ***epigenetic repression*** of the T cell's multipotent developmental potential which can be targeted to improve CAR-T cell efficacy. DNA methylation is a critical regulator of this exhaustion programming. De novo methylation of T-cell plasticity associated genes is coupled to CAR-T cell exhaustion. Deletion of the de novo DNA methyltransferase 3 alpha (DNMT3A) in T cells expressing first- or second-generation CARs universally preserved the cells' ability, in ***IL-10 dependent manner***, to proliferate and mount an antitumor response during prolonged tumor exposure. ***Genome-wide DNA methylation*** profiling defined an atlas of genes targeted for epigenetic silencing including stem-associated genes, TCF7 and LEF1[3] and provides a roadmap for potent CAR-T cell development.

Which mechanisms can improve cell trafficking? In which way can engineered homing receptors help?

Effectiveness of CAR-T in solid tumors is limited by several factors, and perhaps most notably, the trafficking of the CAR-T cell to the tumor itself. Strategies are focused on enhancing CAR-T cell homing to and infiltration into the tumor which can yield therapeutic benefit.

Chemokines mediate lymphocyte homing and migration. Chemokine receptor expressing CAR-T cells show superior cytokine production and T-cell activation/cytotoxicity compared to a CAR-T construct alone. Both osteosarcoma (OS) and rhabdomyosarcoma (RMS) cells significantly increase expression of the chemokine IL-8 after clinically achievable doses of radiation, but not at rest. Since CAR-T cells do not express the receptor for IL-8, construct with an IL-8 receptor (CXCR2) expressing B7H3 CAR was synthesized to improve CAR-T homing in sarcoma.[4] INF-γ production was equivalent between the B7H3 CAR-T2a-CXCR2 T cells and B7H3 CAR-T cells, but IL-2 production was significantly higher in the dual expressing CAR-T cells. Sarcoma tumor bearing mice treated with B7H3 CAR-T2a-CXCR2 T cells, resolved the tumor completely by 4–5 weeks and had long-lasting remission. In another study iPSCs were precisely engineered to co-express CAR and CXCR2 and subsequently differentiated to T cells to generate iPSC-derived CAR-T cells (CAR-iT cells). Like their primary CAR-T cell counterparts, functional chemotaxis of CXCR2+ CAR-iT cells was observed in response to recombinant IL-8. Importantly, CXCR2 expression did not limit CAR-dependent cytolytic function or specificity of CAR-iT cells, underscoring the potential of this approach to enhance CAR-T cell trafficking to tumor site ***(Fate Therapeutics)***.[5] Rational engineering of unique chemokine receptors to deliver the ideal chemokine/chemokine receptor match between tumors and effector cells can be leveraged to enhance tumor targeting and trafficking of CAR-iT cells for more effective treatment of solid tumors.

What are the less explored negative immune regulators and resistance mechanisms that should be targeted?

While decreased CAR expression, cytotoxicity, and TH1 cytokine production are the causes of reduced anti-tumor functions, certain candidates are underexplored such as negative immune regulators PTR1 and PTR2. The knockout of PTR was hypothesized to increase CAR-T cell cytokine activity, phenotype, persistence, and tumor control. Preclinical experiments validated the hypothesis. PTR KO CAR-T cells demonstrated enhanced tumor burden control and extended survival in solid tumor xenograft model compared to mock PTR KO CARTs.[6]

CRISPR screen identified loss of IFNγR signaling and downstream adhesion as a resistance mechanism to CAR-T cell cytotoxicity in solid but not liquid tumors.[7] To systematically identify resistance pathways in solid tumors, ***a genome-wide CRISPR knockout screen in glioblastoma*** cells was created. Glioblastoma cell line U87 was used and targeted endogenously expressed EGFR with CAR-T cells generated from 6 normal donors for the screen. Loss of genes in the interferon gamma receptor (IFNγR) signaling pathway (IFNγR1, JAK1, JAK2) rendered U87 cells resistant to CAR-T cell killing in vitro. IFNγR1 knockout tumors also showed resistance to CAR-T cell treatment in vivo in a glioblastoma line U251 orthotopic model. Resistance to CAR-T cell cytotoxicity through loss of IFNγR1 applied more broadly to solid

tumors as CAR-T cells against IL13Ralpha2 and pancreatic cell lines targeted with either mesothelin or EGFR CAR-T cells also showed this resistance mechanism. However, loss of IFNγR signaling did not impact sensitivity of liquid tumor lines (leukemia, lymphoma, or multiple myeloma) to CAR-T cells in vitro or in an orthotopic model of leukemia treated with CD19 CAR. Glioblastoma cells lacking IFNγR1 had lower upregulation of cell adhesion pathways compared to wild-type glioblastoma cells after exposure to CAR-T cells.

Overexpression of ***canonical AP-1 factor cJun*** prevents CAR-T cell exhaustion and ***improves anti-tumor potency in vivo***, however, its clinical utilization is limited by potential for transformation and oncogenic risk. CAR-T engineered to conditionally express the canonical AP-1 factor cJun increased expansion potential similar to CAR-T cells engineered to constitutively express the cJun transgene, however, the context-dependent upregulation of cJun will limit the risk of oncogenic transformation ***(Gilead)***.[8]

What are the considerations for tumor targets?

A key consideration to develop effective adoptive cell therapy is selection of target against which potent and specific immune response can be elicited. Cancer testis antigens are tumor specific and have shown early promise in TCR T-cell based therapies.

A FIH trial (NCT03686124), enrolled HLA-A*02:01- and PRAME-positive recurrent and/or refractory solid cancer patients, who failed all available standard treatments ***(Immatics)***.[9] TCR-engineered T cells (TCR-T) were directed against an HLA-A*02-restricted peptide derived from the highly prevalent cancer testis antigen PRAME. This target was selected due to ***homogenous expression*** and ***exceptionally high target peptide density per tumor cell***, two features which are critical determinants of anti-tumor activity in TCR-T trials. All evaluable patients (N=12) achieved disease control according to RECIST1.1. Responses were seen in patients with synovial sarcoma (N=3), malignant melanoma (N=2), and head and neck cancer (N=1). ***IMA203 is the first TCR-T product candidate that induced frequent tumor responses across multiple solid cancers*** using transduced T cells at doses below 1 billion and had a manageable safety profile.

What are the new directions in synthetic immunity beyond T and NK cells?

Besides T-cell therapies, the field is also advancing toward harnessing other immune cells e.g. myeloid cells to boost immune responses. Many presentations at SITC shed light on myeloid cell biology, role in immune suppression, novel innate checkpoints (e.g. CD24 on macrophages), and ways to engineer myeloid cells to use as therapeutics akin to adoptive T-cell therapies.

Premetastatic niche is enriched in immune-suppressive genes in myeloid cell cluster. Using syngeneic RMS mice model, researchers showed that genetically engineered myeloid cells (GEMys) can be harnessed as a platform to deliver anti-tumor factor to reprogram metastatic microenvironment.[10] IL12 expressing GEMys shifted the transcription program in premetastatic lung and led to upregulation of genes causing adaptive immune activation and antigen processing and presentation. IL12 GEMys reversed the immune suppressive program, activated T, NK and dendritic cells and effector functions, reduced spontaneous metastases to lung, and led to increased survival. Neoadjuvant injection of IL12 GEMys limited metastases, 90% mice were cured over a year after treatment and remained disease free. IL12 GEMys induce T-cell memory response that protected from tumor challenge and improved efficacy of tumor specific T cells indicating potential of combination with T-cell therapy.

References

1. E. Breman, A. S. Walravens, I. Gennart, et al. Armoring NKG2D CAR T cells with IL-18 improves in vivo anti-tumor activity [Abs#107]. In: SITC 2021; Nov 10-14; Washington DC. Available at: https://jitc.bmj.com/content/9/Suppl_2/A118.
2. Q. Dong, W. Yin, P. Jiang, et al. IL-2 variant improves CAR-T functionality and efficacy against solid tumors [Abs#110]. In: SITC 2021; Nov 10-14; Washington DC. Available at: https://jitc.bmj.com/content/9/Suppl_2/A120.
3. B. Prinzing, C. C. Zebley, C. T. P. Fan, et al. Deleting DNMT3A in CAR T cells prevents exhaustion and enhances antitumor activity. Sci. Translational Med 2021;13(620). Available at: https://www.science.org/doi/10.1126/scitranslmed.abh0272.
4. J. Lake, K. Winkler, A. Harrant, et al. Co-opting IL-8 to enhance efficacy of B7H3 CAR T cells against pediatric sarcoma [Abs#125]. In: SITC 2021; Nov 10-14; Washington DC. Available at: https://jitc.bmj.com/content/9/Suppl_2/A134.
5. M. Hosking, S. Shirinbak, J. Grant, et al. Chemokine receptor engineering enhances trafficking and homing of primary and iPSC-derived CAR-T cells to solid tumors [Abs#120]. In: SITC 2021; Nov 10-14; Washington DC. Available at: https://jitc.bmj.com/content/9/Suppl_2/A129.
6. D. Mai, O. Johnson, C. June. Genetic disruption of negative immune regulators to enhance CAR-T efficacy against solid tumors [Abs#131]. In: SITC 2021; Nov 10-14; Washington DC. Available at: https://jitc.bmj.com/content/9/Suppl_2/A140.
7. R. Larson, M. Kann, S. Bailey, et al. CRISPR screen identifies loss of IFNγR signaling and downstream adhesion as a resistance mechanism to CAR T-cell cytotoxicity in solid but not liquid tumors [Abs#221]. In: SITC 2021; Nov 10-14; Washington DC. Available at: https://jitc.bmj.com/content/9/Suppl_2/A234.
8. Z. Yang, F. Marincola. Context-dependent reversible modulation of cJUN expression by CAR T cells for cancer treatment [Abs#154]. In: SITC 2021; Nov 10-14; Washington DC. Available at: https://jitc.bmj.com/content/9/Suppl_2/A164.
9. M. Wermke, A. M. Tsimberidou, A. Mohamed, et al. Safety and anti-tumor activity of TCR-engineered autologous, PRAME-directed T cells across multiple advanced solid cancers at low doses – clinical update on the ACTengine® IMA203 trial [Abs#959]. In: SITC 2021; Nov 10-14; Washington DC. Available at: https://jitc.bmj.com/content/9/Suppl_2/A1009.
10. S. Kaczanowska, D. Beury, H. Qin, et al. Genetically engineered myeloid cells (GEMys) as a platform to enhance antitumor immunity [Abs#209]. In: SITC 2021; Nov 10-14; Washington DC. Available at: https://jitc.bmj.com/content/9/Suppl_2/A220.

***SmartAnalyst is helping clients interpret the data presented at SITC 2021 focusing on key developments in cell and gene therapy in solid tumors.* Contact us to learn more**

Please Contact:
John.L'Ecuyer@smartanalyst.com

GLOBAL HEADQUARTERS
(New York, USA)
285 Madison Ave
22nd Floor, New York
Ny 10017
Mobile: +1 (646) 852-5097

EUROPE
(London, Uk)
1.04 Power Road Studios
114 Power Road, Chiswick
London W3 5PY
Mobile: +44-(0)-752-163-8242
Mobile: +44-(0)-772-574-4296

ASIA PACIFIC
(Gurgaon, INDIA)
First Floor, DLF Plaza Tower
DLF Qutub Enclave, DLF City
Phase-I, Gurgaon 122002
Haryana, India
Tel: 91-124-4313800