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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

A Lenalidomide-Inducible Suicide Switch for Gene- and Cell-Based Therapy

William Lin[1,2,3], *Ditsa Sarkar, PhDMSc*[3,4,2], *Joanna Y Kim*[3,4,2], *Nelson H Knudsen, PhD*[5,6,4], *Isabel C Lane*[2,3,4], *Andi Cheng*[5,4,6], *Gabriele Kembaun*[4,2,3], *Tamina Kienka*[4,3], *Jeannie Carreiro*[5,7], *Amanda A Bouffard*[4,3], *Marcela V Maus, MD PhD*[8,3,9], *Robert T Manguso, PhD*[5,4,6], *Max Jan, MDPhD*[2,4,3,5]

[1] Cancer Center, Massachusetts General Hospital, Charlestown, MA
[2] Department of Pathology, Massachusetts General Hospital, Boston, MA
[3] Harvard Medical School, Boston, MA
[4] Cancer Center, Massachusetts General Hospital, Boston, MA
[5] Broad Institute of MIT and Harvard, Boston, MA
[6] Department of Medicine, Massachusetts General Hospital, Boston, MA
[7] Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA
[8] Blood and Marrow Transplant Program, Massachusetts General Hospital, Boston, MA
[9] Cellular Immunotherapy Program, Cancer Center, Massachusetts General Hospital, Boston, MA

Cellular immunotherapies, including chimeric antigen receptor (CAR) T cells, have demonstrated promising efficacy against hematologic malignancies and liquid tumors but run the risk of severe life-threatening toxicities. Moreover, further modifications to enhance cellular immunotherapy potency are being tested to overcome treatment failures due to T cell dysfunction or suppression in the tumor microenvironment. Consequently, for novel and/or highly active cellular immunotherapies the inclusion of "suicide switches" may be prudent to suppress treatment-related adverse events by inducing specific depletion of the engineered cells. The clinical deployment of existing cell therapy suicide switches has been limited in part by imperfect performance characteristics, reliance on non-FDA-approved controller drugs, and the potential immunogenicity of non-human sequences. Herein, we report the engineering of a clinically suitable cell therapy suicide switch activated by targeted protein degradation, and the credentialing of this system to rapidly and irreversibly deplete CAR T cells *in vitro* and *in vivo*.

We and others have previously demonstrated that lenalidomide-inducible degron tags can be linked to proteins of interest to allow for their selective degradation (Jan et al, Science Translational Medicine, 2021; Carbonneau et al, Cell Chemical Biology, 2021; Koduri et al, PNAS, 2019). Lenalidomide acts as a "molecular glue" that recruits neosubstrate proteins to the CRL4CRBN E3 ubiquitin ligase to be ubiquitinated and subsequently degraded by the proteasome. Caspase-activated DNase (CAD) and its inhibitor (ICAD) are broadly expressed pro- and anti-apoptotic proteins, respectively. ICAD serves as a chaperone for CAD folding and sequesters CAD activity by forming an inactive heterodimer with CAD. Upon apoptosis signaling, activated Caspase 3 cleaves ICAD, liberating CAD to form an active homodimer that acts as a pair of "molecular scissors" to create double strand breaks in the genome. We hypothesized that the overexpression of CAD and an ICAD-degron fusion protein at a stoichiometric 1:1 ratio could be well-tolerated, and furthermore, that lenalidomide treatment would deplete the ICAD-degron protein, thereby freeing CAD to cause cell death.

After iterative optimization of promoter strength, transgene order, and degron placement, we evaluated an optimized ICAD-degron-CAD lenalidomide suicide switch versus inducible Caspase 9 in primary human T cells. The lenalidomide suicide switch induced more complete cell depletion versus iCasp9, and maximal cell depletion was seen with subtherapeutic nanomolar concentrations of lenalidomide. In multi-day co-culture live cell imaging assays with co-transduced and sorted CAR suicide switch T cells, tumor cell cytolysis and CAR T cell proliferation were comparable with or without expression of the suicide switch. Lenalidomide addition rapidly inhibited tumor cell cytolysis and depleted the suicide switch CAR T cells without subsequent re-expansion. In an *in vivo* NSG murine xenograft model with JeKo-1 tumor cell engraftment, suicide switch and control CAR T cells demonstrated comparable anti-tumor activity. For the suicide switch CAR T cells, tumor expansion accelerated after pomalidomide treatment, consistent with engineered cell suppression after suicide switch induction. We have also co-delivered the CAR and 2.2 kilobase suicide switch as a single multi-cistronic lentivector.

In summary, a lenalidomide-inducible suicide switch composed of the stoichiometric pair of CAD and ICAD-degron proteins enabled rapid, near-complete engineered cell depletion without interfering with CAR T cell growth or effector functions in the

models tested. Composed of all-human sequences and a controller available as a generic drug, the lenalidomide-inducible suicide switch is a clinically suitable system that may have broad applications to safeguard the development of highly potent investigational cellular immunotherapies.

Disclosures Maus: *Oncternal:* Current equity holder in private company; *TCR2:* Current equity holder in private company; *Neximmune:* Current equity holder in private company; *Novartis:* Patents & Royalties; *Promab:* Patents & Royalties; *Genocea:* Current equity holder in private company; *2SeventyBio:* Consultancy; *Century Therapeutics:* Current equity holder in private company; *Massachusetts General Hospital:* Patents & Royalties. **Manguso:** *Calico Life Sciences:* Research Funding; *Related Sciences:* Consultancy, Current equity holder in private company; *Bristol Myers Squibb:* Consultancy. **Jan:** *RA Ventures:* Consultancy; *Massachusetts General Hospital:* Patents & Royalties.

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