

## HLA-INDEPENDENT T CELL RECEPTORS EFFECTIVELY TARGET LOW ABUNDANCE ANTIGENS

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**Background** Chimeric antigen receptors (CARs) engage antigen independently of HLA and enable sustained T cell proliferation when they are endowed with both activating and costimulatory functions. While remission rates have been noticeably elevated in numerous clinical trials targeting CD19, CD22 or BCMA, relapses are common. One of the several underlying relapse mechanisms is antigen escape, which refers to a relapsing tumor that is either negative for the targeted antigen or expresses the latter at a low level. Failure to eliminate antigen-low tumors raises questions about the sensitivity of CARs and the minimum antigen density that is required for effective tumor eradication. Unlike CARs, TCRs engage antigen in an HLA-dependent manner, and they do so with high sensitivity. We hypothesized that a TCR/CD3 complex containing the same heavy and light immunoglobulin chains as a CAR will display increased sensitivity to the target antigen.

**Methods** We edited the TRAC locus in human primary T cells to establish a novel antigen receptor structure, termed HLA-independent TCR or HIT receptor, by incorporating into the TCR/CD3 complex the same heavy and light chains as those of a corresponding CAR. We assessed their antigen sensitivity against a panel of cell lines expressing different antigen levels, analyzing their cytotoxicity, cytokine secretion, signaling response and degranulation activity. HIT and CAR T cells were further evaluated for their anti-tumor response using established ALL and AML mouse models.

**Results** CD19-TRAC-HIT and CD19-TRAC-CAR T cells lysed wild-type NALM6 (~27,000 CD19 molecules) and NALM6 variants with 100-fold less CD19. As CD19 levels decreased further, CAR T cells no longer killed their target, in contrast to HIT T cells. HIT T cells showed increased expression of IFN-gamma, IL-2 and TNF-alpha upon exposure to NALM6 cells expressing ~20 CD19 molecules per cell, compared to CAR T cells. This increased sensitivity of HIT receptors correlated to their greater signaling response, upon exposure to the low-antigen-density NALM6. Phospho-proteomic analyses further confirmed this increased response of HIT T cells to low antigen levels. Altogether, these results confirm that HIT receptors endow T cells with greater antigen sensitivity than canonical CARs. We further showed that HIT T cells have higher in vivo anti-tumor activity compared to CAR T cells in mice bearing low-antigen-density ALL or AML.

**Conclusions** HIT receptors consistently afford high antigen sensitivity and mediate tumor recognition beyond what current CARs can provide. HIT receptors open new prospects for targeting cell surface antigens of low abundance.

**Ethics Approval** Eight- to 12-week-old NOD/SCID/IL-2Rgamma-null (NSG) male mice (Jackson Laboratory) were used under a protocol approved by the MSKCC Institutional Animal Care and Use Committee.

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