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703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Novel, Engineered, Modified Fcγ Receptor I (CD64)-Expressing T Cells (SolidT) Combined with Rituximab Exhibited Profound Anti-Tumor Activity and Memory in a CD20+ Raji Xenograft Model of Lymphoma

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Background: CD19 CART cell therapy has been a major advance in the treatment of lymphoma, but is limited due to profound toxicity and relapse, in part due to loss of CD19 expression on malignant cells. Following our discovery of FcγRI (CD64)-expressing T cells in tumors and inflamed tissues (PMID 31449054), we developed novel, engineered cytotoxic T cells ("SolidT") with increased specificity, reduced exhaustion, and attenuated cytokine secretion compared to other engineered cell therapies (e.g., CAR-T, CAR-NK). SolidT are produced by transducing a retroviral vector encoding an enhanced FcγRI into T cells for use with tumor-targeting monoclonal antibodies (mAbs). SolidT activation correlates with surface tumor antigen density. Unlike CART, where tonic signaling causes premature exhaustion and limited tumor infiltration, SolidT are not exhausted prematurely. They are activated and persist only by mAb bound to tumor-specific antigen at sufficient density, and they take on a T effector memory (T_{EM}) phenotype. We previously showed that SolidT + mAb has anti-tumor activity in vitro and in vivo in several tumor models, including immunodeficient HER2 xenografts and an immunocompetent syngeneic B16-TYRP1 model (PMID 37070661). To further assess the SolidT platform, we examined the effect of SolidT + rituximab (R) in vitro and in vivo in a CD20+ Raji-Luciferase (Luc) xenograft model.

Methods: In *in vitro* Raji-Luc and Daudi-Luc models, SolidT + R was compared to CD19 CART, R + sham transfected T cells (Sham), Sham only, and SolidT only. Cytotoxicity was measured by flow analysis of Annexin V and/or Luc assay. IFNγ and Granzyme B were measured by ELISA. Addition of intravenous immunoglobulin (IVIG; 5mg/ml) to cytotoxicity co-cultures was used to evaluate the effect of non-specific IgGs on the efficacy of the combination treatment. *In vitro* safety was evaluated in a similar manner using primary cultures of kidney and lung epithelial cells. To assess the anti-tumor activity of SolidT, NSG mice were inoculated intravenously (IV) with $1x10^6$ Raji-Luc cells (n=8). SolidT ($1x10^7$ cells) was given on Day (D) 1. R (250 mg) was given on D1 and then weekly (125 mg). Mice were monitored for weight, clinical signs, and survival until D41 when the experiment was terminated to conduct endpoint analyses including gross pathology, histopathology, and $CD3^+$ immunohistochemistry (IHC).

Results: *In vitro*, SolidT + R showed higher cytotoxicity and attenuated cytokine levels against Raji-Luc compared to CD19 CART cells. Similar results were obtained in a Daudi-Luc model. Addition of IVIG did not activate SolidT cells, and though IFNγ secretion was attenuated, cytotoxicity was not affected. The *in vitro* co-culture of SolidT cells with primary epithelial cells demonstrated no activation of the effector cells and no death of primary target cells. *In vivo*, mice treated with SolidT + R had increased survival and reduced tumor burden against Raji-Luc compared to mice treated with R alone (Figure 1). On pathology, 5/8 SolidT + R mice had no tumors detected and 3 had only abdominal tumors. R mice developed tumors in the lungs (2/8), liver (7/8), and abdomen (4/8). Bone marrow immunophenotyping revealed that the presence of T_{EM} phenotype highly correlated to better survival and lower tumor burden. Gross pathology analysis revealed no treatment-related pathology. IHC showed increased inflammation, lymphocyte infiltration, and necrosis in tumors from SolidT + R-treated mice, as well as intense infiltration of $CD3^+$ human cells in the tumors at end point D41. The *in vivo* Raji-Luc model was performed twice with similar results.

Conclusions: Consistent with prior results in solid tumor models targeting HER2 or TYRP1, these data demonstrated that the SolidT platform could be effectively targeted against CD20+ cell lines. A single administration of SolidT cells in combination with R prevented tumor formation and prolonged survival in a CD20+ Raji-Luc model. This experiment demonstrated that marrow-resident SolidT cells developed a T_{EM} phenotype that positively correlated with outcome. These data confirm that the SolidT platform can be targeted against different tumor antigens by using different tumor-targeting mAbs. The SolidT

platform could represent a paradigm shift in the approach to engineered cellular therapy for hematological malignancies and solid tumors.

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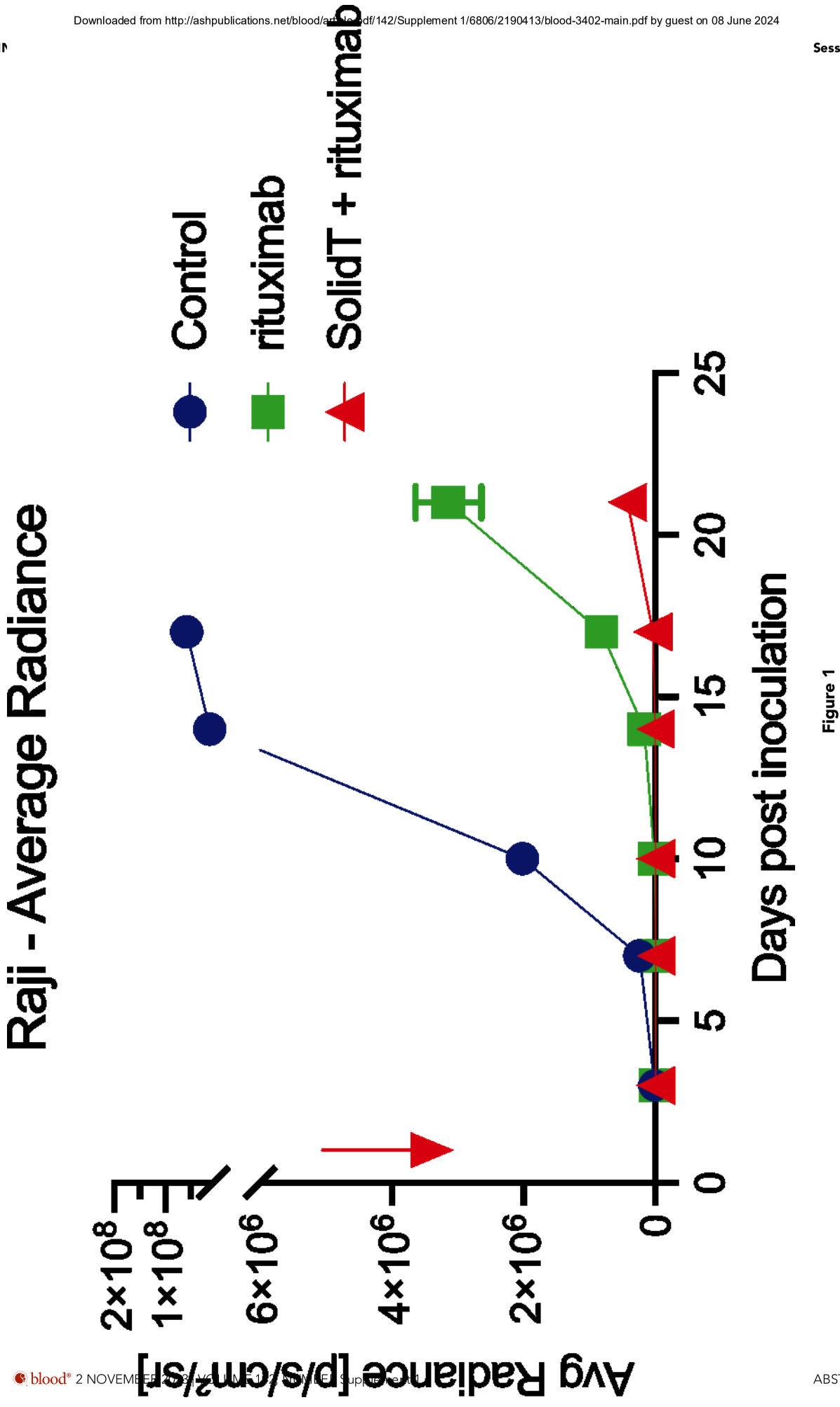

Figure 1