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ORAL ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

IL1RAP-Specific T Cell Engager (TCE) Antibody Efficiently Depletes Acute Myeloid Leukemia (AML) Leukemic Stem Cells (LSCs)

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LSCs are self-renewal primitive cells that initiate and maintain AML. Because they are highly therapy resistant, they are often responsible for treatment failures. Due to the lack of a specific antigen (Ag) and their quiescent nature, LSCs have been challenging to target and eliminate. IL1RAP is highly expressed on most human AML cell lines and primary blasts, including LSC-enriched CD34+CD38- cells, but not on normal hematopoietic stem cells (HSCs). To leverage this observation, we generated over 50 unique mouse IL1RAP monoclonal (m) antibodies (Abs), grouped them based on sequence, and selected representative members from the clades to generate murine-human chimeric mAbs. One of the mAbs, termed #24, had the strongest antibody-dependent cell-mediated cytotoxicity (ADCC) properties and the greatest in vivo anti-AML activity. Thus, it was advanced to generate a bispecific (Bs) anti-IL1RAP/CD3 TCE Ab termed BiF002, which incorporates a human IgG1 Fc mutated to lack FcR binding. Incubation of BIF002 with healthy donor T cells and AML cell lines or primary AML blasts induced IL1RAP dependent T cell activation, and Ab-dependent T cell lysis of leukemic cells in a dose-, time- and effector to target (E:T) ratio-dependent manner. The IC_{50} against MOLM13 and THP-1 was subnanomolar (0.047nM, 95%CI:0.036-0.060nM and 0.025nM, 95%CI:0.14-0.40nM, respectively), at E:T ratio 5:1 at 48 hours using resting T cells. The IC_{50} of $IL1RAP^{pos}$ AML bulk and CD34+ blasts (n=6) was also subnanomolar (0.45nM; 95%CI: 0.28-1.96nM). Co-cultures of normal CD34+ cells with BIF002 and T cells did not activate T cells or eliminate the normal CD34+ cells.
To test the antileukemic activity of BIF002 in vivo, we engrafted luciferase (luc)-expressing MOLM13 cells into NSG-SGM3 (NSGS) mice. On day (D) 3, in vitro expanded healthy donors' T cells (3x10 ^{6}/mouse, weekly), with either BIF002 or a control IgG (10μg,) were administered intravenously (IV) every 3 days x 4-weeks. Mice treated with T cells + BIF002 exhibited a 100-fold greater tumor reduction on D33 than other groups, as measured by bioluminescence imaging (BLI), and had a significantly longer overall survival (OS; median 54.5 D) compared to IgG (27 D, p=0.0045), BIF002 alone (36 D, p=0.0049), or T cells + IgG (33 D, p=0.0062) treated controls. In initial dose finding studies using a similar schema, lower BIF002 doses (0.1 and 1 μg) were tested. Mice treated with T cells and 1 μg of BIF002 had a 100-fold tumor reduction on D29 and longer OS (median 57 D) compared to T cells + 0.1μg of BIF002 (31 D, p=0.0018) or T cells + vehicle (34 D, p=0.0019). We also engrafted luc-expressing primary blasts from a relapsed/refractory complex karyotype AML patient into NSGS mice that treated for 3 weeks using the above described schema (10μg). In this experiment, we used as control BIF026, a bispecific Ab (BsAb) carrying point mutations in both Fab arms to abrogate target binding to IL1RAP and CD3. Mice treated with T cells + BIF002 exhibited a >1000-fold reduction in disease burden and longer OS than BIF026 or BIF002 alone or T-cells+ BIF026-treated mice (median 46 D vs 26, 25 or 30 D, respectively, all comparisons, p=0.0003). In a second patient-derived xenograft (PDX) model, blasts from a relapsed complex karyotype AML patient were engrafted into NSG mice. Treatment was initiated on D10, with T cells given weekly along with 10μg of BIF002 or BIF026 for 3 weeks. After 70 days, all mice treated with T cells + BIF002 were alive, with no

detectable blasts in peripheral blood or bone marrow (BM), while all vehicle (median 38 D, p=0.0002) and T cells + BIF026 (38 D, p=0.0001) treated controls had died of leukemia. To determine if T cells + BIF002 eradicated LSCs, we randomly selected 3 female mice from each group on D 34 and transplanted BM mononuclear cells (MNC) into secondary NSGS recipients (n=7). At D 90, no recipients of BM from donors treated with T cells + BIF002 had detectable blasts and remained alive, while all vehicle (median 26 D, p=0.0004) and T cells + BIF026 (median 26 D, p=0.0002) treated controls succumbed to AML. Importantly, no obvious hematopoietic or non-hematologic side effects were observed after BIF002 administration alone or with activated T cells in normal and leukemic mice.

In summary, we provide evidence of the subnanomolar potency and efficacy of BIF002, a novel anti-IL1RAP/CD3 TCE, in eliminating AML blasts, including LSCs. BIF002 is currently undergoing IND-enabling studies.

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