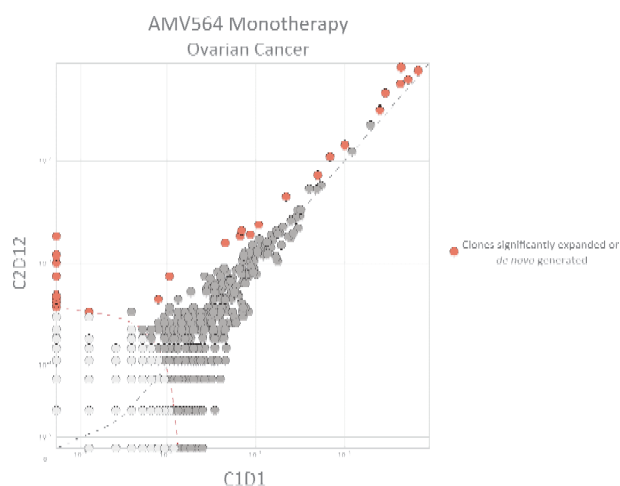
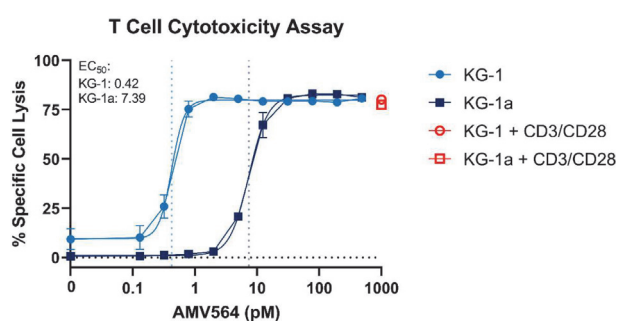


indicate that one cycle of treatment is sufficient to expand and generate de novo clones (figure 2). We developed a primary cell cytotoxicity assay and observe that cytotoxic potency is target dependent. Target cell killing and T cell activation/proliferation depend on CD33 clustering, and both CD4 and CD8 T cells can engage and kill target cells. This is illustrated in assays with KG-1 (M2, clustered) and KG-1a (M0, not clustered) cell lines, in which the KG-1 cells have an EC₅₀ 15–20 fold lower than the M0 cell line (figure 3). In addition, there is little to no detectable binding or killing of monocytes or neutrophils, which is consistent with the absence of neutropenia in patients enrolled in the trial to date.



Abstract 692 Figure 2 TcRb CDR3 sequencing of an ovarian cancer patient shows extensive clonal expansion upon treatment. Scatter plots represent clonal abundance in the periphery between Baseline (C1D1) and C1D12 using the differential Abundance tool (Adaptive Biotechnology)



Abstract 692 Figure 3 Cytotoxicity assay using fresh primary T cells demonstrates target selectivity on two cell lines with equivalent CD33 surface expression. T cell activation by AMV564 at clinically relevant doses is equivalent to CD3/CD28 stimulation

Conclusions AMV564 is a potent conditional T cell agonist which is clinically active. We demonstrate that the combination of T cell activation, increased T cell diversity, and target specificity allow AMV564 to deplete MDSCs and restore a native immune response to cancer.

Ethics Approval This study was approved by the Institutional Review Board (IRB) or Independent Ethics Committee (IEC) at each participating institution.

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693 APX601, A NOVEL TNFR2 ANTAGONIST ANTIBODY FOR CANCER IMMUNOTHERAPY

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Background A key barrier to effective immunotherapy for cancer is the immunosuppressive tumor microenvironment (TME) characterized by infiltrating regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSC). While depletion of immune-suppressive cells is a promising cancer immunotherapy strategy, current approaches are ineffective due to lack of specificity and safety concerns. Tumor Necrosis Factor Receptor 2 (TNFR2) is emerging as a novel, selective target to overcome immunosuppression in TME. TNFR2 expression is generally restricted to highly immunosuppressive cell populations in the TME and the TNFR2-TNF- α pathway plays an important role in the generation and survival of these cells. TNFR2 is also an oncogene upregulated on certain tumors and can enhance tumor cell survival. Thus, targeting TNFR2 is a promising therapeutic approach with multiple potential mechanisms of action.

Methods A diverse panel of antibodies to TNFR2 was created using APXiMAB™, Apexigen's proprietary rabbit monoclonal antibody technology. A robust assessment of over 100 antibody candidates for TNFR2 binding, TNF- α blockade and functional assays yielded APX601, a humanized IgG1 antibody, as the lead therapeutic candidate. The ability of APX601 to reverse immune suppression was assessed in Treg and MDSC suppression assays. In addition, the ability of APX601 to deplete TNFR2-expressing Treg and tumor cells was assessed both in vitro and in vivo using the mouse Colo205 xenograft model.

Results APX601 binds specifically to human TNFR2 with high affinity (K_d = 47 pM) and recognizes a unique epitope in the CRD1 domain of TNFR2. APX601 is a potent antagonist that blocks the TNFR2-TNF- α interaction in cell-based ligand binding assays (IC₅₀ = 0.149 nM). APX601 is capable of reversing immune suppression via two mechanisms: 1) significant blockade of the immunosuppressive functions of both Tregs and MDSCs by inhibiting the binding of TNFR2 to its ligand TNF- α and 2) depletion of TNFR2-expressing Tregs, MDSC and tumor cells via antibody-dependent cell cytotoxicity (ADCC) (EC₅₀ = 1.14 nM) and ADCP (EC₅₀ = 0.71 nM) effector functions.

Conclusions APX601 is a potent TNFR2 antagonist antibody that reverses immune suppression by targeting TNFR2-expressing Treg and MDSC, and induces killing of tumor cells. Our data support the further development of APX601, a promising immunotherapeutic antibody with multiple potential mechanisms of action, for the treatment of a variety of solid tumors.

Ethics Approval Healthy human blood samples were obtained from Stanford Blood Center (Palo Alto, CA) from consenting donors under an approved protocol.

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694 NC410 IS A NOVEL IMMUNOMEDICINE FOR THE TREATMENT OF SOLID TUMORS

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Background Abnormalities in the extracellular matrix of tumor microenvironments support tumor progression, lead to