by enhancing adhesion and transmigration of T cells to endothelial (HUVEC) cells. AT1412 was most potently enhancing transendothelial T-cell migration, in contrast to a high affinity version of AT1412 or other high affinity anti-CD9 reference antibodies (e.g. ALB6). Enhanced immune cell infiltration is also observed in immunodeficient mice harbouring a human immune system (HIS). AT1412 strongly enhanced CD8 T-cell and macrophage infiltration resulting in tumor rejection (A375 melanoma). PD-1 checkpoint blockade is further sustaining this effect. In a second melanoma model carrying a PD-1 resistant and highly aggressive tumor (SK-MEL5) AT1412 together with nivolumab was inducing full tumor rejection, while either one of the antibodies alone did not.

Conclusions The safety of AT1412 has been assessed in preclinical development and is well tolerated up to 10 mg/kg (highest dose tested) by non human primates. AT1412 demonstrated a half-life of 8.5 days, supporting 2–3 weekly administration in humans. Besides transient thrombocytopenia no other pathological deviations were observed. No effect on coagulation parameters, bruising or bleeding were observed macro- or microscopically. The thrombocytopenia is reversible, and its recovery accelerated in those animals developing anti-drug antibodies. First in Human clinical study is planned to start early 2021.

Ethics Approval Study protocols were approved by the Medical Ethical Committee of the Leiden University Medical Center (Leiden, Netherlands).

Consent Blood was obtained after written informed consent by the patient.

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722 INHIBITION OF INTEGRIN AVß8-MEDIATED TGF-ß ACTIVATION WITH C6D4 PROVIDES IMPROVED POTENCY AND SELECTIVITY VS GENERAL TGF-ß INHIBITORS FOR CANCER IMMUNOTHERAPY

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Background TGF-ß plays a key role in immune evasion as a critical regulator of both innate and adaptive tumor immunity and promotes broad immunosuppressive effects on numerous inflammatory cell subpopulations ultimately resulting in tumor immune tolerance and evasion.1 It has also been implicated in resistance to immune checkpoint therapies, and additive or synergistic effects of dual TGF-ß and PD-1 inhibition has been reported.2 3 A number of TGF-ß inhibitors are in clinical development with different modes of action. Most protein-based inhibitors are designed to block diffusible TGF-ß from interacting with its proximal signaling receptor TGF-ßR2 and includes monoclonal antibodies (Mabs) and receptor traps. This investigation compares inhibition of TGF-ß by a number of inhibitors and the integrin avß8 (C6D4) to assess their relative potential as cancer therapeutics.

Methods No reporter system currently exists to investigate the mechanistic basis of cell-intrinsic TGF-ß activation, whereby the L-TGF-ß presenting cell is also the cell that responds to TGF-ß signaling (figure 1). To build a cell-intrinsic TGF-ß activation system, TMLC cells were stably transfectected with wild-type (WT) TGF-ß. Without co-transfecting GARP, TMLC do not present L-TGF-ß on their cell surface. When co-transfectect with TGF-ß and GARP, high levels of cell surface expression of L-TGF-ß are detected. Additionally, to build a cell-intrinsic TGF-ß activation system which express a non-releasable form of TGF-ß, we mutated the L-TGF-ß furin cleavage site (R249A) and similarly expressed the L-TGF-ß (R249A)/GARP complex on the surface of TGF-ß reporter cells (TMLC). These cell-intrinsic TGF-ß activation systems were used to assess the relative abilities of Mabs avß8, TGF-ß, TGF-ßR2, GARP or TGF-ßR2 receptor trap to inhibit avß8-mediated TGF-ß activation.

Results avß8 exhibited superior inhibitory activity compared with other TGF-ß inhibitors, which was similar in both diffusible and non-diffusible models (figure 2). The biologic relevance of these finding was confirmed using CD4+ T-cells in place of the reporter cells where TGF-ß-dependent Treg generation was almost completely blocked by avß8 but was poorly inhibited by the other TGF-ß inhibitors.
Conclusions In this study avß8 exhibited dramatic TGF-ß inhibitory activity compared with a wide range of inhibitors in development. Because integrin avß8 may direct TGF-ß signaling from within its latent complex, this may offer an advantage for target specificity and avoid the challenges faced by non-specific TGF-ß inhibitors. These findings characterize avß8 as a novel and potent immunotherapy drug for further clinical investigation.

Trial Registration NA
Ethics Approval NA

REFERENCES


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LYMPH NODE-TARGETED AMP-VACCINE ENABLES TUMOR-DIRECTED MKRAS-SPECIFIC IMMUNE RESPONSES WITH POTENT POLYFUNCTIONAL AND CYTOTOXIC ACTIVITY
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Background Subunit vaccines targeting tumor antigens have shown limited capacity for expanding cytotoxic T-cells against tumors in the clinic. Especially in the case of KRAS-driven tumors, responses elicited by conventional vaccines have been exceedingly weak. For molecular immunogens including peptides and oligonucleotides, inefficient delivery to immune cells residing in the lymphatics is a significant challenge limiting their ability to induce cancer-directed immune responses of sufficient strength and functionality to impact tumors. Improving the targeting of immunogens to lymph nodes (LN), where resident immune cells potentally orchestrate immunity, can substantially amplify their ability to induce effective tumor-directed immunity. Here, we demonstrate such an approach for significantly enhancing mKRAS-directed T-cell responses by precisely targeting antigens and adjuvants directly to the draining LN through a simple one-step conjugation to albumin-binding lipids. These amphiphilic conjugates (‘Amphiphiles’, AMP) then ‘hitch-hike’ on albumin into the LNs where they elicit strong immune responses. LN accumulation of structurally optimized amphiphiles in mice is greatly improved over soluble equivalents.

Methods C57BL/6J mice received two or more doses of benchmark or amphiphile-modified vaccines, comprised of mKRAS peptide and CpG adjuvant, subcutaneously injected into the tail base in two-week intervals. Immunological readouts were performed 7 days post dosing. For ELISPOT analysis of IFNγ and Granzyme B production and flowcytometric bead array analysis of Th1/2 cytokines, splenocytes were harvested and re-stimulated with antigen overnight. In vivo, cytolytic capabilities of antigen-specific T-cells were evaluated by pulsing CFSE-stained splenocytes from naïve mice with mKRAS antigen and injecting these cells intravenously into immunized mice. Recovery of CFSE-labeled target cells from immunized mice was performed 24h later and analyzed flowcytometrically.

Results We show robust immune responses that yield strong activation against all common mutations in the mKRAS protein compared to low or undetectable responses generated by soluble or benchmark treatments. Further, this response is composed of CD8+ as well as CD8+ T-cells resulting in the production of high levels of Th1-associated cytokines upon re-stimulation with mKRAS-specific peptides in vitro. In vivo, robust cytolytic function towards mKRAS-presenting targets can be measured in T-cells.

Conclusions By targeting immunogens directly and precisely to the LNs, the Amphiphile platform can significantly amplify the potency of subunit vaccines. In the case of mKRAS, substantially improved cytolytic immune responses represent a promising therapeutic strategy for targeting mKRAS-driven tumor growth and survival in a large fraction of human tumors. Furthermore, this platform technology is simple, rapid and scalable for broad clinical application.

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724 STAT3 INHIBITION IN ACUTE MYELOID LEUKEMIA CELLS ALLOWS FOR TLR9-DRIVEN DIFFERENTIATION TO IMMUNOGENIC MONOCYTIC CELLS AND INDUCTION OF T-CELL MEDIATED IMMUNE RESPONSES
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Background Signal transducer and activator of transcription factor 3 (STAT3) is commonly activated in acute myeloid leukemia (AML) and known for supporting cancer cell proliferation and survival. Recently, we demonstrated that STAT3 also plays a critical role ensuring AML immune evasion. Intravenous injections of bi-functional decoy oligodeoxyribonucleotides (CpG-STAT3dODN) blocked STAT3 activity and induced TLR9 signaling in Cbbl/MYH11/Mpl (CMM) AML cells, thereby resulting in immunogenic effects and T cell-mediated immune responses and leukemia regression.

Methods To understand the molecular mechanisms of the CpG-STAT3 decoy- induced AML differential and immunogenicity, we performed global gene expression analysis on the in vivo treated AML cells using oligonucleotide strategy as well as an inducible STAT3 gene silencing.

Results Transcriptional profiling revealed the upregulation of myeloid cell differentiation related genes, such as IfI8, Cebpa, and Gad45A with reduction of oncogenic Runx1 and Run1t1 in CMM leukemic cells after CpG-STAT3dODN but not after control treatments. CpG-STAT3dODN treatment also upregulated set of antigen-presentation related genes, such as CIta, Il12a, and Ifng in CMM AML cells. Importantly, the induction of IfI8 and Cebpa, with the concomitant suppression of Runx1 were found specifically in the subset of differentiated CD11b+ CMM cells but not in the bulk CD11b– leukemic cells. These effects were likely related to epigenetic reprogramming of AML cells as indicated by treatment-induced changes in the expression and protein levels of STAT3 regulated DNA methyltransferases, DNMT1 and DNMT3a/b. Furthermore, our initial studies suggest that STAT3 inhibition/TLR9 activation leads to immunogenic effects also in a xenotransplanted model of human FLT3-ITD MV4-11 leukemia in humanized mice. CpG-STAT3dODN alone or together with clinically-relevant demethylating agent (Decitabine) triggered differentiation of MV4-11 cells into CD11b+HLA-DR+CD86+ antigen-presenting cells (APCs) and increased ratio of CD8+ to