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List of Abbreviations

Allo-HSCT	Allogeneic hematopoietic stem cell transplantation
APOE	Apolipoprotein E
COVID-19	Coronavirus disease 2019
CSC	Cancer stem cells
DAMP	Damage-associated molecular pattern
eQTL	Expression quantitative trait locus
GVHD	Graft versus host disease
GVL	Graft versus leukemia
GVT	Graft versus tumor
LXR	Liver-X-receptor
MDS	Myelodysplastic syndromes
MDSC	Myeloid-derived suppressor cells
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
scRNA-seq	Single cell RNA-sequencing
SNP	Single nucleotide polymorphism
TCGA	The Cancer Genome Atlas
TME	Tumor microenvironment
Tregs	Regulatory T cells

1 Introduction

1.1 The promise of precision medicine

Precision medicine refers to the tailoring of healthcare to the individual. While the concept of precision medicine is not new, recent advances in targeted therapies and high-dimensional diagnostics, particularly using systems biology approaches, have sparked hope that we are entering a period where precision medicine may become reality for most patients (Ashley, 2016).

The evolution of sequencing technologies over the last 20 years since the publication of the first sequenced human genome has likely been the most significant advance towards precision medicine: for example, next-generation sequencing technologies allow for the economical sequencing of genomes from patient cohorts to identify subgroups of patients that derive benefit from specific treatments (Sosman *et al.*, 2012). The need for precision medicine approaches is particularly high in oncology, given the pronounced heterogeneity of malignant diseases even within the same tumor entity (Hoadley *et al.*, 2014).

Notable examples of early breakthroughs in precision oncology include the use of the tyrosine kinase inhibitor imatinib in patients with chronic myeloid leukemia (O'Brien *et al.*, 2003) and the use of anti-EGFR therapy in colorectal cancer depending on *KRAS* mutation status (Karapetis *et al.*, 2008). Despite these advances, precision medicine remains elusive for the majority of patients. Reasons for the lack of widespread benefit from precision medicine include a lack of known actionable targets: it is estimated that only 5-10% of all cancer patients benefit from targeted therapies based on genetic assessment (Marquart *et al.*, 2018). In addition, the use of targeted therapies for specific genetic alterations often achieves only short-term response, as exemplified by patients receiving *BRAF*-inhibitors in *BRAF*-mutant malignant melanoma (Robert *et al.*, 2019). Orthogonal assessment of a

patient's disease, including metabolic, epigenetic and proteomic profiling as well as single cell sequencing readouts may provide for novel therapeutic angles.

This work describes five studies that investigate different facets contributing to the realization of precision medicine: In the first study, we investigated the molecular basis of how the activation of a specific transcription factor shapes the tumor microenvironment in an anti-tumor fashion. In the two subsequent studies, we assessed the contribution of common germline variants of the *APOE* gene shaping pro-metastatic phenotypes in melanoma and immunity in COVID-19, thereby establishing *APOE* genotype as a potential biomarker in these contexts. In the fourth study, we assessed the use of uric acid levels as a predictor for a common immunologic complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT), graft versus host disease (GVHD). In the final study, we investigated the hematopoietic hierarchy and aberrant expression patterns on stem and progenitor cell populations in the bone marrow of patients with myelodysplastic syndromes (MDS) to identify novel potential therapeutic targets.

1.2 Cancer immunotherapy

Immunotherapy has transformed the treatment of several cancer types. The identification of CTLA4 and PD1 as immune "checkpoints" that physiologically restrain T cell priming and activation and the subsequent development of antibody therapies for their inhibition marked a breakthrough in cancer medicine (Leach *et al.*, 1996; Hodi *et al.*, 2010; Freeman *et al.*, 2000; Topalian *et al.*, 2012). Combination therapy with CTLA4 and PD1 inhibition achieves approximately 50% long-term remission in patients with advanced melanoma (Larkin *et al.*, 2019), dramatically improving the outcome of this patient population. In addition to CTLA4- and PD1-inhibition, the inhibition of PD-L1 has entered clinical use (Mariathasan *et al.*, 2018).

Mechanistically, the action of CTLA4 and PD1 inhibition is based on the suppression of molecular checkpoints that restrain T cell activity. The two checkpoints act at different stages of T cell activation. CTLA4 competes with the co-stimulatory receptor CD28 for binding to CD80 and CD86 expressed by antigen-presenting cells, thereby modulating

the interaction between T cells and antigen-presenting cells during priming. While CD28 ligation leads to activation of T cell signaling, CTLA4 ligation inhibits T cell activation and proliferation (Brunet *et al.*, 1987). Therapeutic inhibition of CTLA4 therefore unleashes T cell activation. Additionally, CTLA4 inhibition leads to depletion of tumor-promoting T regulatory cells, potentially contributing to its anti-tumor activity (Peggs *et al.*, 2009).

Similar to CTLA4, PD1 is also expressed on T cells. The main ligands of PD1 are PD-L1 and PD-L2, which are expressed by multiple cell types upon exposure to pro-inflammatory cytokines (Francisco *et al.*, 2010). Activation of PD1 activates inhibitory signaling in T cells. While CTLA4 signaling takes place mostly in the lymph nodes, PD1 signaling predominantly occurs in peripheral sites of T cell activity, such as in the tumor microenvironment in the context of anti-tumor immunity. Given the temporal and spatial differences in how CTLA4 and PD1 impact T cell function, inhibition of CTLA4 and PD1 complement each other and act additively in the therapeutic setting (Larkin *et al.*, 2019).

Inhibition of PD-L1 blocks the interaction of PD1 with PD-L1 similar to PD1 inhibition. However, the effects of PD1 and PD-L1 inhibition are not interchangeable, since PD-L1 inhibition does not abrogate the interaction of PD1 with PD-L2 and because PD-L1 can also bind to B7 proteins (Butte *et al.*, 2007; Obeid *et al.*, 2016; Lakins *et al.*, 2018).

Several other immune checkpoints have been identified, many of which are being assessed for therapeutic targeting, such as LAG-3, TIM-3, TIGIT, and VISTA (Qin *et al.*, 2019). However, none of these have entered standard clinical practice yet. In addition to checkpoint-targeting immunotherapies, adoptive T cell therapies, including the administration of ex-vivo expanded tumor-infiltrating T cells, T cell receptor-transduced T cells, and chimeric antigen receptor T cells, have shown major promise (Rosenberg and Restifo, 2015). However, their use is limited to certain hematological malignancies owing to the challenge in identifying tumor-specific targets in solid cancers (Leko and Rosenberg, 2020).

Unfortunately, most patients with advanced cancers do not derive benefit from current immunotherapy regimens, rendering a better understanding of the mechanisms underlying the heterogeneity of clinical response an urgent clinical need. Several factors have been found to correlate with response to immunotherapy. Perhaps most importantly, it has been established that tumoral mutational burden (a surrogate parameter for the neoantigen

repertoire of a tumor) correlates with improved outcome (Snyder *et al.*, 2014; Van Allen *et al.*, 2015). In addition, the expression of the PD1 ligand PD-L1 and DNA mismatch repair capacity of the tumor have been shown to correlate with immunotherapy outcome (Topalian *et al.*, 2012; Davis and Patel, 2019; Le *et al.*, 2015). More generally, the pre-existing immune landscape in a given tumor, also referred to as the immune contexture, correlates with response (Bruni *et al.*, 2020; Binnewies *et al.*, 2018). In the tumor microenvironment, three major classes of immune states have been described: immune-desert tumors are mostly void of immune cells. In immune excluded tumors immune cells are present but mostly localized at the tumor margins, while inflamed tumors exhibit infiltration of the tumors with cytotoxic immune cells. Not surprisingly, immune desert (also termed immunologically cold) tumors show the least response to immunotherapy, while inflamed (or immunologically hot) tumors show the best response (Mariathasan *et al.*, 2018).

Several additional factors have been found to modulate the interplay between the immune system and cancer. These include the microbiome (Zitvogel *et al.*, 2018) and the degree of tumor heterogeneity (McGranahan *et al.*, 2016; Riaz *et al.*, 2017). Notably, oncogenic signaling in the cancer cells contributes to shaping the tumor microenvironment, as exemplified by WNT/beta-Catenin signaling suppressing the recruitment of specific subsets of dendritic cells (Spranger *et al.*, 2015).

In light of the potential efficacy of immunotherapy on the one hand and the large fraction of patients that do not benefit from current regimens on the other hand, it remains an urgent clinical need to identify novel biomarkers predicting for therapy response as well as to identify novel treatment approaches.

1.3 Germline variation in cancer biology

Germline variation is well established as an important modulator of the risk for the development of certain tumors. For example, mutations in the tumor suppressor genes *BRCA1* and *BRCA2* increase the risk of breast and ovarian cancers (Miki *et al.*, 1994; Wooster *et al.*, 1995).

In contrast to its role in tumorigenesis, the role of germline variation in modulating the progression of a tumor once it has formed is only beginning to be appreciated (Huang *et al.*, 2018; Chatrath *et al.*, 2021). Few reports link specific germline variants with cancer outcome: for example, *FCGR3A* and *P2RX7* variants were found to be associated with outcome of melanoma and breast cancer, respectively (Arce Vargas *et al.*, 2018; Ghiringhelli *et al.*, 2009). Several more examples exist, although in all of these cases the identified association was correlative with no assessment of causality (Koessler *et al.*, 2009; Summers *et al.*, 2020; Shu *et al.*, 2018; Marasigan *et al.*, 2019).

Recent studies have begun to assess the impact of germline variation on cancer outcome more systematically (Chatrath *et al.*, 2021). For example, Chatrath and colleagues assessed the impact of exome-wide germline variants on the survival of more than 10,000 patients of The Cancer Genome Atlas (TCGA) (Chatrath *et al.*, 2020). Notably, approximately half of the variants associated with tumor progression were in genes that had previously been implicated in tumorigenesis, while the rest were in genes not previously found to act as tumor suppressors or oncogenes. In a different study, Carter and colleagues reported on the role of germline variants in shaping the somatic mutational landscape a tumor acquires (Carter *et al.*, 2017). A clinically salient result of this finding is that germline variants in mismatch repair genes enhance the generation of neoantigens and are thereby associated with enhanced response to immunotherapy (Arora *et al.*, 2019).

Germline variants can potentially exert effects on tumor progression by acting on tumor cells directly, such as modulating susceptibility to cell death or altering signaling pathways regulating other phenotypes (Skowronska *et al.*, 2012; Ulaganathan *et al.*, 2015). Importantly, germline variants can also modulate tumor progression by acting on other components of the tumor microenvironment (TME), such as on immune cells (Kogan *et al.*, 2018). Notably, analyses of genotype-phenotype associations have revealed multiple single nucleotide polymorphisms (SNP) to be associated with immune function (Parkes *et al.*, 2013; Lee *et al.*, 2014; Raj *et al.*, 2014; Ye *et al.*, 2014; Cho and Feldman, 2015; Tian *et al.*, 2017). Recent studies have harnessed single cell RNA-sequencing (scRNA-seq) to determine the role of SNPs in modulating the expression of genes as so-called expression quantitative trait loci (eQTL) (Yazar *et al.*, 2022; Perez *et al.*, 2022).

In light of the findings above it seems plausible that germline variation participates in shaping tumor progression, potentially in part by modulating anti tumor immunity. Consistent with this assumption, maximal heterozygosity at HLA-I loci was shown to correlate with improved response to cancer immunotherapy (Chowell *et al.*, 2018). In addition, analyses of the TCGA uncovered correlations of many germline variants with distinct immune profiles in the TME (Sayaman *et al.*, 2021; Shahamatdar *et al.*, 2020; Lim *et al.*, 2018). However, scarce data exist causally linking individual germline genotypes with differential outcome in cancer.

1.4 COVID-19

In early 2020, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the cause of the global coronavirus disease 2019 (COVID-19) pandemic. Over the next 2.5 years, more than 6 million people succumbed to COVID-19 and several billion people got infected.

SARS-CoV-2 is an RNA betacoronavirus that enters cells via binding of its spike protein to the angiotensin-converting enzyme 2 (ACE2) receptor (Zhou *et al.*, 2020). It is transmitted primarily via aerosols and respiratory droplets with a median incubation time of 2-5 days depending on the variant (Lamers and Haagmans, 2022). Frequent symptoms of COVID-19 include cough, anosmia, fever, myalgia and diarrhae, but severe illness ensues in some individuals with dyspnoea and respiratory failure.

In patients with severe disease, immune dysregulation and hyperactivation appear to be a common phenomenon, with marked elevations of pro-inflammatory cytokines present in the serum (Chen *et al.*, 2020a). SARS-CoV-2 may also lead to extrapulmonary manifestations and symptoms, although for many of these it remains unclear whether they are a direct consequence of infection (Puelles *et al.*, 2020).

The highly heterogeneous outcome of SARS-CoV-2 infection with clinical presentations ranging from asymptomatic infection to fatal disease has been a particularly challenging characteristic in dealing with the pandemic. Some factors predicting for adverse outcome quickly became apparent during the early stages of the pandemic, including advanced age,

male sex, and the presence of autoantibodies to Interferon signaling (Williamson *et al.*, 2020; Zhang *et al.*, 2022). However, these factors only partially explain the pronounced heterogeneity of COVID-19 outcomes. The germline genetic makeup was postulated to contribute to differential clinical course of COVID-19, and several reports have associated germline variants with differential outcomes in COVID-19 (Zhang *et al.*, 2022). Amongst these, candidate gene approaches have identified rare variants in genes implicated in Interferon signaling to correlate with detrimental outcomes in COVID-19 (Zhang *et al.*, 2020; Asano *et al.*, 2021). In addition, several genomic regions were associated with poor COVID-19 outcomes in genome-wide association studies (The Severe Covid-19 GWAS Group, 2020; Pairo-Castineira *et al.*, 2021a; Nakanishi *et al.*, 2021). However, the epidemiological nature of these impressive efforts has left it unknown whether germline genetic variation causally modulates SARS-CoV-2 infection or whether it is merely correlatively associated with differential outcomes.

Modeling disease in animal models remains the gold standard to assess the causality of given variables. In the case of SARS-CoV-2, the wildtype variant of the virus proved inefficient at infecting rodents due to differences in ACE2, the main receptor mediating cellular uptake of the virus. This represented a major roadblock to mechanistic studies in mouse models (Zhou *et al.*, 2020). However, several methods have been developed to enable modeling of COVID-19 in mice: K18-hACE2 mice, expressing human ACE2 driven by the cytokeratin-18 promoter, were shown to be efficiently infected by wildtype SARS-CoV-2 (Zheng *et al.*, 2021). Similarly, adenoviral transduction of lung epithelia of wildtype mice with human ACE2 rendered these mice susceptible to SARS-CoV-2 infection (Israelow *et al.*). In a third approach, the laboratory of Ralph Baric derived a variant of SARS-CoV-2 by serial in-vivo passaging that evolved to efficiently infect wildtype mice (Dinnon *et al.*, 2020; Leist *et al.*, 2020). One major advantage of this latter approach is the feasibility to use this model across different genetic backgrounds. Despite the availability of these models, so far no germline variants have been tested using in-vivo models for a causal impact on COVID-19 outcomes.

Overall, a comprehensive understanding of why certain individuals are susceptible to adverse outcomes upon SARS-CoV-2 infection is lacking. The identification of biomarkers for

the prediction of COVID-19 outcomes would hold the potential to improve preventative and therapeutic measures, including early booster vaccinations as well as early administration of antiviral therapies.

1.5 The LXR/APOE pathway

Apolipoprotein E (APOE) is a secreted glycoprotein with canonical roles in lipid metabolism: the presence of APOE on lipoprotein particles facilitates their uptake into cells, primarily hepatocytes. In addition to its role in organismal metabolism, APOE plays roles in several other processes, including immune modulation (Martinez-Martinez *et al.*, 2020).

The impact of APOE on immunity is complex and seems to be context-specific: *Apoe*-knockout mice exhibit increased susceptibility to different infections with viruses and intracellular bacteria (Ludewig *et al.*, 2001; Martens *et al.*, 2008; Toledo *et al.*, 2015). However, *Apoe*-deficient mice were also shown to exhibit increased $CD4^+$ T cell activation due to enhanced MHCII-mediated antigen presentation (Bonacina *et al.*, 2018).

The expression of the *APOE* gene encoding APOE is regulated by liver-X-receptors (LXR) (Evans and Mangelsdorf, 2014). LXRs belong to the nuclear receptor family of ligand-dependent transcription factors. Oxysterols constitute the main endogenous ligands for LXRs, and activation of LXRs leads to the transcription of a gene program mediating uptake, transport, and metabolism of cholesterol. LXRs also participate in the regulation of fatty acid metabolism (Hong and Tontonoz, 2014).

1.5.1 LXR-APOE signaling in cancer

In cancer, melanoma cells with high metastatic capacity were shown to downregulate APOE in order to abrogate the inhibitory effect of APOE on key tumor progression and metastasis phenotypes, including cancer cell invasion and endothelial recruitment (Pencheva *et al.*, 2012). Mechanistically, these anti-tumoral effects are mediated by APOE binding to the LRP1 receptor on tumor cells to inhibit invasion and by binding to the LRP8 receptor on endothelial cells to inhibit endothelial recruitment. In animal models, APOE was found

to also suppress progression of breast and ovarian cancers (Alikhani *et al.*, 2013; Lai *et al.*, 2018).

Pharmacologic activation of LXRs, the transcriptional activators of APOE, using a synthetic LXR agonist was shown to suppress melanoma progression and metastasis in an APOE-dependent manner (Pencheva *et al.*, 2014).

1.5.2 Germline variants of *APOE*

An important aspect of APOE biology is the existence of three highly common variants that are distinct from one another in just one or two amino acids: the most common *APOE3* allele is defined by a cysteine and an arginine in positions 112 and 158, respectively; the other two highly common variants, *APOE2* and *APOE4*, instead have cysteins or arginines at both positions, respectively. While these sequence difference are subtle, they confer important pathophysiological consequences: the *APOE4* variant is the biggest monogenetic risk factor for Alzheimer's disease, while the *APOE2* variant is protective (Strittmatter *et al.*, 1993; Corder *et al.*, 1994). *APOE* variants were also shown to impact other phenotypes, including cardiovascular disease risk and longevity (Mahley, 2016; Bennet *et al.*, 2007; Xu *et al.*, 2016; Deelen *et al.*, 2019).

The major impact of these variants on the biological function of APOE is likely attributable to their impact on protein folding, which in turn gives rise to differential binding affinities to at least some of the several known APOE receptors (Chen *et al.*, 2011; Weisgraber *et al.*, 1982; Xian *et al.*, 2018).

Limited data suggest that *APOE* genotype also modulates immune responses in infectious disease. Individuals carrying at least one copy of *APOE4* showed increased levels of activated circulating T cells (Bonacina *et al.*, 2018). Similarly, *APOE4* carriers showed higher cytokine levels upon LPS challenges relative to *APOE3* homozygotes (Gale *et al.*, 2014), and *APOE4* microglia exhibited increased activation upon LPS exposure (Shi *et al.*, 2017). Interestingly, in cryptosporidial infection, *APOE4* mice were protected against severe disease (Azevedo *et al.*, 2014). In humans, *APOE4* genotype correlated with favorable outcome in hepatitis C infection (Wozniak *et al.*, 2002; Mueller *et al.*, 2016) and women with *APOE4* were protected from infection in contexts of high pathogen exposure (van Exel *et al.*, 2017). Despite these

data, the role of *APOE* genotype in modulating infectious disease outcome remains to be more comprehensively determined.

1.6 Hematopoietic stem cell transplantation

The transplantation of hematopoietic stem cells from an unrelated or related donor (allogeneic hematopoietic stem cell transplantation, allo-HSCT) provides a potentially curative therapeutic option for many patients with hematological diseases that are otherwise uncurable. The high therapeutic efficacy of allo-HSCT is based on two factors: first, repletion of hematopoetic stem cells enables the administration of high-dose, myeloablative chemo- and/or radiotherapy. The second effect is based on an allogeneic immune reaction of the transplanted cells against cancer cells (graft-versus-tumor effect, GVT).

Unfortunately, allo-HSCT can give rise to several complications. In addition to infections and cancer relapse, immunologic reactions against non-tumor cells, so-called graft versus host disease, constitutes a major reason for the high morbidity and mortality in allo-HSCT patients (Zeiser and Blazar, 2017).

Given the beneficial effect of GVT on the one hand and the detrimental effect of GVHD on the other, careful adjusting of pharmacological immunosuppression is paramount in patients undergoing allo-HSCT. Some biomarkers exist that can predict the risk of GVHD, including serum levels of soluble ST2 and REG3α (Hartwell *et al.*, 2018; Xiao *et al.*, 2013). However, the identification of further biomarkers is required to enable their use for guiding clinical management of allo-HSCT patients.

1.7 Myelodysplastic syndromes

Myelodysplastic syndromes (MDS) comprise a group of related diseases that are characterized by dysplasia of myeloid cells and inefficient hematopoiesis. The incidence of MDS is highest in the elderly. MDS carries a risk of progression into acute leukemia. While low-risk cases only require supportive therapy, higher-risk cases require treatment with

chemotherapy or hypomethylating agents, with allo-HSCT constituting the only curative option (Cazzola, 2020).

Recent advances have provided evidence for the existence of cancer propagating cancer stem cells (CSC) in MDS (Nilsson *et al.*, 2000; Pang *et al.*, 2013; Will *et al.*, 2012; Woll *et al.*, 2014). To improve the efficacy of MDS therapy, there is a major clinical need to identify novel treatment targets. Targeting of CSCs would be particularly desirable, since their depletion may be essential and sufficient for cancer elimination (Woll and Jacobsen, 2021). Multiple markers have been shown to be aberrantly expressed in myelodysplastic bone marrow cells (Chung *et al.*, 2017; Chen *et al.*, 2019). However, it remains insufficiently understood whether these candidate targets are expressed by CSCs as opposed to more mature progenitor cells and whether their targeting will efficiently deplete CSCs.

2 Original research

2.1 APOE as a modulator of immunity in cancer and infectious disease

2.1.1 APOE enhances anti-tumor immunity via depletion of myeloid-derived suppressor cells

LXR/ApoE activation restricts innate immune suppression in cancer.

Tavazoie MF, Pollack I, Tanqueco R, Ostendorf BN, Reis BS, Gonsalves FC, Kurth I, Andreu-Agullo C, Derbyshire ML, Posada J, Takeda S, Tafreshian KN, Rowinsky E, Szarek M, Waltzman RJ, Mcmillan EA, Zhao C, Mita M, Mita A, Chmielowski B, Postow MA, Ribas A, Mucida D, and Tavazoie SF (2018).

Cell 172:825–840.e18.

DOI: https://doi.org/10.1016/j.cell.2017.12.026

Apolipoprotein E (APOE) is a protein with pleiotropic roles in lipid metabolism and immunity. APOE was previously shown to suppress melanoma progression and metastasis by interfering with cancer progression phenotypes, including cancer invasion and angiogenesis (Pencheva *et al.*, 2012). Importantly, the anti-tumoral effect of APOE can be harnessed therapeutically by pharmacological activation of Liver-X-receptors (Pencheva *et al.*, 2014). In this work, we asked whether in light of the known role of APOE in modulating immunity in other contexts, it also impacts the immune response to cancer (Ludewig *et al.*, 2001; Martens *et al.*, 2008; Toledo *et al.*, 2015; Angeli *et al.*, 2004; van den Elzen *et al.*, 2005; Bonacina *et al.*, 2018).

Using syngeneic mouse models of melanoma and lung cancer, we found that activation of LXR receptors using synthetic agonists led to a pronounced depletion of myeloid-derived suppressor cells (MDSC), an immune cell subset promoting tumor progression (Shipp *et al.*, 2016). Epistasis experiments revealed this effect to be mediated by APOE, since LXR-activation in *Apoe*-knockout mice failed to elicit MDSC depletion and suppression of tumor progression. In-vitro experiments showed that APOE induced MDSC apoptosis through binding to the APOE receptor LRP8. In vivo, LXR/APOE-mediated depletion of MDSC enhanced the activity of anti-tumor cytotoxic T cells. Additionally, LXR-agonistic therapy enhanced the efficacy of adoptive T cell therapy in a syngeneic melanoma model as well as efficacy of immune checkpoint blockade. Notably, in a phase 1 clinical trial in humans, patients administered LXR agonistic therapy recapitulated depletion of MDSC and activation of cytotoxic T cells.

The results of this study suggest LXR activation as a novel immunotherapeutic approach targeting immunosuppressive myeloid cells. Our combination therapy studies indicate that LXR agonism may be additive or synergistic to established treatment approaches, including adoptive T cell transfer and immune checkpoint blockade.

Several questions arise from this work: Given the large interindividual heterogeneity of MDSC infiltration between tumors it remains unclear whether the LXR/APOE axis can be therapeutically harnessed in individuals with negligible MDSC infiltration. In addition, given the evidence of LXR signaling to play roles in immune modulation beyond MDSC depletion, it remains unclear whether additional molecular mechanisms contribute to the anti-tumor effect of LXR activation.

Most importantly, given the presence of three highly common variants of *APOE* in the human population and their known impact on several phenotypes as outlined above (see 1.5.2), this work in conjunction with previous work on the newly established role of APOE in modulating tumor progression raises the question of whether different APOE variants differentially modulate anti-tumor immunity and tumor progression. We followed up on this question in the work described in the next section (see 2.1.2).

2.1.2 Germline variants of *APOE* modulate key pro-metastatic melanoma phenotypes

Common germline variants of the human *APOE* gene modulate melanoma progression and survival.

Ostendorf BN, Bilanovic J, Adaku N, Tafreshian KN, Tavora B, Vaughan RD, and Tavazoie SF (2020).

Nature Medicine 26:1048–1053.

DOI: https://doi.org/10.1038/s41591-020-0879-3

The study described above revealed that transcriptional activation of APOE inhibits tumor progression and metastasis by impacting several pro-tumoral phenotypes. Given the occurence of three common variants of *APOE* in the human population, we next addressed the question of whether *APOE* genotypic variation imparts differential impacts on tumor progression and metastasis.

In the study referenced above we found that *APOE* genetic variation impacted melanoma progression and metastasis by modulating several key tumor progression phenotypes. Transplantation of C57BL6/j-syngeneic YUMM1.7 mouse melanoma cells into transgenic mice models harboring one of the three human *APOE* variants in place of murine *Apoe* (Knouff *et al.*, 1999; Sullivan *et al.*, 1997, 1998) resulted in markedly different tumor progression. Strikingly, mice with the Alzheimer's predisposition *APOE4* allele showed the slowest tumor progression, while mice with the Alzheimer's protective *APOE2* allele exhibited most rapid melanoma progression. Of note, this was also true in a genetically engineered melanoma mouse model (Adaku *et al.*, 2022).

We found that in addition to modulating anti-tumor immunity, APOE variants exerted their effect on tumor progression by differentially impacting two main phenotypes required for tumor progression, tumor cell invasion and endothelial recruitment. Incubation of melanoma cells genetically silenced for endogenous *Apoe*-expression were incubated with recombinant APOE variants and their invasive potential was assessed in a matrigel invasion chamber. Strikingly, the APOE4 variant had a more pronounced effect on inhibiting

melanoma cell invasion relative to APOE2, indicating that this effect may contribute to the different rates of metastasis we observed in mice bearing the *APOE4* and *APOE2* alleles.

In addition, recombinant APOE4 had a higher capacity at inhibiting endothelial recruitment in a melanoma/endothelial cell co-culture system, indicating that APOE4 may have a higher capacity at inhibiting tumor angiogenesis. Consistent with this, tumor vascularization was significantly sparser in tumors hosted by *APOE4* relative to those in *APOE2* mice.

In more recent work, we showed that in-vivo, APOE2 seems to exert a gain-of-function phenotype by enhancing tumor cell translation (Adaku *et al.*, 2022). To which extent this phenotype contributes to the impact of *APOE* genotype on modulating tumor progression remains to be assessed.

2.1.3 Common variants of *APOE* are causal modulators of outcome in COVID-19

Common human genetic variants of *APOE* impact murine COVID-19 mortality

Ostendorf BN, Patel MA, Bilanovic J, Hoffmann HH, Carrasco SE, Rice CM, and Tavazoie SF (2022).

Nature 611:346–351.

DOI: https://doi.org/10.1038/s41586-022-05344-2

The COVID-19 pandemic has had a devastating impact on human health with millions of deaths by late 2022. One of the characteristic features of COVID-19 is the large interindividual spectrum of clinical outcomes, ranging from completely asymptomatic infection to severe disease and death. While the mechanisms underlying the heterogeneity of COVID-19 outcomes remain incompletely understood, many studies have shown associations between germline variants and outcome (Zhang *et al.*, 2022; COVID-19 Host Genetics Initiative *et al.*, 2021). However, it remained unclear whether common genetic variations are causally (as opposed to just correlatively) involved in modulating the outcome of COVID-19.

In this study we assessed whether common germline variants of *APOE*, shown above to modulate anti-tumor immunity (Ostendorf *et al.*, 2020), impact the course of SARS-CoV-2 infection. Using a mouse model of COVID-19, we found that both mice with the *APOE2* and the *APOE4* genotype showed significantly worse survival outcomes relative to mice with the most common *APOE3* allele. *APOE2* and *APOE4* mice exhibited increased viral load early after infection, and transcriptional and flow cytometric immune profiling of the lungs of infected mice showed blunted adaptive immunity relative to *APOE3* mice, a feature known to be associated with adverse outcomes in COVID-19 in humans (Lucas *et al.*, 2020; Mathew *et al.*, 2020). Notably, a large single cell RNA-seq dataset that we generated to assess the immunological ramifications of *APOE* genotype on COVID-19 response suggested immune hyperactivation in *APOE2* but not in *APOE4* mice. Consistent with the immune response diverging between these two genotypes during later infection stages, *APOE4* mice showed a pronounced increase of virus-specific $CD8^+$ T cells on day 11 post infection relative to *APOE3* and *APOE4* mice. Interestingly, recombinant APOE3, but not recombinant APOE2

and APOE4, was capable of suppressing SARS-CoV-2 infection in-vitro. These data indicate that the effect of *APOE* genotype on COVID-19 is mediated by a combination of its impact on antiviral immunity and on viral infection.

Finally, to assess whether *APOE* genotype also impacts COVID-19 in humans, we analyzed individuals with confirmed SARS-CoV-2 infection in the UK Biobank (Sudlow *et al.*, 2015). Consistent with our animal model data, individuals homozygous for *APOE4* showed significantly reduced survival relative to *APOE3* and *APOE2* homozygotes.

In sum, our study for the first time causally implicates common germline variants in shaping COVID-19 outcomes in an animal model. It warrants future investigation into the role of *APOE* genotype in the outcome of other infectious diseases. In addition, it is desirable to further characterize the molecular mechanisms underlying the impact of *APOE* on antiviral immunity and viral infection.

2.2 Uric acid levels correlate with graft versus host disease

Association between low uric acid levels and acute graft-versus-host disease.

Ostendorf BN, Blau O, Uharek L, Blau IW, and Penack O (2015).

Annals of Hematology 94:139–144.

DOI: https://doi.org/10.1007/s00277-014-2180-3

In the three studies presented above we explore the role of a gene product and its genetic variants in shaping anti-tumoral and anti-viral immunity. In this study, we also explored a potential biomarker for its association with an immunologic outcome, focusing on the association of uric acid with the incidence of graft versus host disease (GVHD) after hematoepoietic stem cell transplantation.

Allogeneic hematopoietic stem cell transplatation (allo-HSCT) offers curative potential for patients with advanced hematological malignancies. The efficacy of allo-HSCT is in part due to the potent graft versus leukemia (GVL) effect. Unfortunately, allo-reactive immunity can target not only malignant cells but can also result in GVHD, characterized by immune activation against healthy tissues. Thus, careful balancing between maximizing GVL while limiting the risk for GVHD is required for successful allo-HSCT. Identifying the factors that predispose for GVHD as a major contributor to post-transplantation morbidity and mortality is a major clinical need.

In this study we assessed the impact of peripheral blood uric acid levels on the incidence of GVHD post allo-HSCT. Uric acid acts as a molecular indicator of tissue damage (damage-associated molecular pattern, DAMP) (Martinon *et al.*, 2006). In our retrospective analysis of uric acid levels and clinical outcome in 228 patients undergoing allo-HSCT, we made the surprising finding that low uric acid levels at the time of transplantation correlated with higher GVHD risk. We did not detect any significant associations between uric acid levels and overall survival and progression-free survival after transplantation.

While these findings were surprising given the known role of uric acid in aggravating inflammation, it has been previously shown that patients with GVHD exhibit reduced

antioxidative capacity. Since uric acid acts as an antioxidant, this offers a potential explanation for the association of low uric acid levels with increased GVHD risk. In light of the retrospective and monocentric nature of our study prospective studies are warranted to confirm these findings.

2.3 Phenotypic characterization of cancer stem cells in myelodysplastic syndromes

Phenotypic characterization of aberrant stem and progenitor cell populations in myelodysplastic syndromes.

Ostendorf BN, Flenner E, Flörcken A, and Westermann J (2018).

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The studies discussed above explored the association and putative mechanistic implication of biomarkers in different immunologic contexts, focusing on biomarkers that predominantly act on the host organism's defense. Here, in contrast, we assessed the presence of surface markers as potential treatment targets on cancer cells in a specific hematopoietic malignancy, myelodysplastic syndrome (MDS).

Myelodysplastic syndromes comprise clonal hematopoietic diseases which are characterized by ineffective hematopoiesis. Several studies have traced the origin of MDS to disease propagating cancer stem cells (Nilsson *et al.*, 2000; Will *et al.*, 2012; Woll *et al.*, 2014). The presence of cancer stem cells is important, since cancer stem cells can be hard to deplete with conventional therapies and can give rise to disease relapse and therapy resistance. Therefore, targeting of cancer stem cells in MDS and other malignancies would mark a major advancement towards specific and lasting therapies.

In this work we undertook a prospective study to phenotypically characterize the stem cell compartment in patients with MDS. Using flow cytometry, we comprehensively examined the hematopoietic hierarchy and expression of candidate markers in 20 patients with MDS and compared them to samples from patients with cytopenia of benign origin or with B-Non-Hodgkin-Lymphoma without bone marrow infiltration. We identified skewing of the hematopoietic hierarchy as well as aberrant expression of surface markers in patients with MDS. Specifically, we found that patients with high-risk MDS (as defined by the presence of excess blasts) exhibited expanded multipotent progenitor cell and and granulocyte

macrophage progenitor cell compartments. Interestingly, not only high- but also low-risk MDS patients showed relative depletion of the myeloid erythroid progenitor cell compartment. In addition, we found early hematopoietic stem cells in MDS to aberrantly express CLL-1, indicating that CLL-1 may be a viable therapeutic candidate to target cancer stem cells in MDS. In addition to these findings, we observed aberrant expression patterns in stem and progenitor cell populations primarily in patients with high- rather than low-risk MDS, including reduced expression of ALDH and increased expression of CD44 and CD47.

The advent of single cell sequencing technologies offers new avenues to complement our findings from this study. In particular, it remains unclear to which degree expression of a given marker such as CLL-1 is preserved on all cancer stem cells and absent from physiological stem cells (Toft-Petersen *et al.*, 2016).

3 Discussion

3.1 LXR/APOE activation as a novel approach to immunotherapy

The majority of patients with advanced cancer do not derive benefit from currently approved immunotherapies, rendering the identification of novel treatment approaches an urgent clinical need. Previous work from the Tavazoie lab implicated the secreted protein APOE in inhibiting key cancer progression phenotypes such as cancer cell invasion and angiogenesis (Pencheva *et al.*, 2012). Importantly, it was shown that upregulation of APOE expression could be harnessed therapeutically by pharmacological activation of Liver-X-receptor transcription factors upstream of APOE expression (Pencheva *et al.*, 2014). The first study described herein assessed whether the anti-tumor activity of the LXR/APOE-axis is partially mediated by the immune system (Tavazoie *et al.*, 2018). Pharmacological activation of LXRs led to robust depletion of myeloid-derived suppressor cells across several tumor models. This in turn unleashed T cell anti-tumor immunity. Mechanistic analyses showed that recombinant APOE induced apoptosis of MDSCs via binding to the LRP8 receptor. As such, LXR activation constitutes a novel approach to enhancing anti-tumor immunity other than the clinically used inhibition of the CTLA4 and PD1/PD-L1 checkpoints, which target the priming and activation of T cells.

After the publication of our results several other studies validated our findings. Wan et al. and Liang et al. observed efficent depletion of MDSCs upon LXR activation in murine breast and lung cancer models, respectively (Wan *et al.*, 2020; Liang and Shen, 2020). In addition, Loeuillard et al. observed potent synergy of LXR agonistic therapy with immune checkpoint blockade in a model of cholangiocarcinoma (Loeuillard *et al.*, 2020). These studies indicate that LXR agonistic therapy may be viable in cancer types beyond the ones studied in our work.

Importantly, LXR signaling has been shown to be active in many different cell types, including several immune cell types (Bensinger *et al.*, 2008; Joseph *et al.*, 2003; Spann and Glass, 2013). In addition, it is known that the myeloid cell landscape in the TME of different tumor types and across different patients varies substantially (Gabrilovich, 2017). Therefore, it remains an important open question whether LXR agonistic therapy elicits other anti-tumor effects in addition to MDSC depletion and whether it remains effective in tumors that exhibit low MDSC infiltration. A recent study observed a marked reduction of tumor-promoting regulatory T cells (Tregs) upon pharmacologic LXR activation (Carbo *et al.*, 2021). This effect depended on host rather than tumoral expression of LXRs, although it remains to be determined whether the depletion of Tregs was directly mediated by LXR activation (and if so in which cell type) or secondary to other effects on the TME. It is desirable that future studies dissect the mechanistic effect of pharmacologic LXR activation by cell type-specific deletion of LXRs and by performing epistasis experiments with depletion of specific immune cell types. Adding to the complexity, it remains unclear to which degree both LXRα and LXRβ contribute to the effect of LXR agonistic therapy, although the synthetic agonist used in our work exhibits markedly higher affinity to LXRβ.

Of note, other groups have also assessed non-immunological sequelae of administering LXR agonists for anti-cancer therapy (Lin and Gustafsson, 2015). For example, several studies observed an anti-proliferative and pro-apoptotic effect of LXR activation on cancer cells (Chen *et al.*, 2020b; Rudalska *et al.*, 2021). However, the lack of therapeutic efficacy in LXR double-knockout mice in our study and in the study of Carbó *et al.* indicates that these effects do not play major roles in the model systems assessed by us (Tavazoie *et al.*, 2018; Carbo *et al.*, 2021). It seems conceivable that the mechanism of action of LXR-agonistic anti-tumor activity depends on the tumor type and potentially other yet unknown factors.

In conclusion our work demonstrated that LXR agonistic therapy offers the potential to augment anti-tumoral immunity. Its mechanism of action is orthogonal to established immunotherapies, such as immune checkpoint inhibition, by targeting immunosuppressive myeloid cells in the tumor-microenenvironment rather than directly unleashing T cell activity. Currently ongoing studies based on these results test the clinical tolerability and efficacy of LXR agonistic anti-cancer therapies in humans (clinical trial ID NCT02922764).

3.2 The role of germline variation in tumor progression and outcome

Germline variants have long been established as modulators of tumor incidence, but their role in modulating cancer progression and outcome is only beginning to be appreciated (Chatrath *et al.*, 2021). Our study showing a major impact of *APOE* germline variants on melanoma progression and outcome suggests that the role germline variants play in modulating tumor progression is currently underappreciated.

Our study raises several questions regarding the specific role of *APOE* variants in cancer progression. First, prospective studies will need to validate the impact of *APOE* genotype on melanoma outcome to pave the way for potential clinical applications of our findings. Our observation of an association in certain immunotherapeutic settings, but potentially not in others, indicates that the impact of *APOE* genotype may be context-specific. It would be desirable to identify the factors that potentially interact with this association and thus promote context-specificity.

In addition, the molecular mechanisms through which *APOE* genotype modulates anti-melanoma immunity remain to be more comprehensively determined. Given our findings on the capacity of APOE to induce apoptosis in tumor-promoting MDSCs via binding to the LRP8 receptor described above (Tavazoie *et al.*, 2018), one intriguing hypothesis is that different APOE variants induce MDSC apoptosis to different degrees due to differential binding affinities to the LRP8 receptor.

Regarding the molecular mechanism of how APOE germline variation modulates cancer cell invasion and endothelial recruitment, it is likely that in line with previous work from the Tavazoie laboratory (Pencheva *et al.*, 2012), the LRP1 and LRP8 receptors on cancer and endothelial cells, respectively, mediate these effects. It would be desirable to formally address this hypothesis by performing epistasis experiments with LRP1- or LRP8-knockout cell lines in the context of treatment with different APOE variants.

Furthermore, it remains unclear how differential engagement of LRP1 and LRP8 signaling is molecularly mediated downstream. LRP1 is a large endocytic receptor that is known to exhibit binding affinity to more than 40 ligands (Kanekiyo and Bu, 2014). It is conceivable

that the effect of APOE variants on LRP1 signaling is either mediated through a direct impact on LRP1 signaling or by altering the availability of LRP1 for binding to other ligands. Intriguingly, we recently found that different APOE variants differentially regulate cellular translation through LRP1 signaling (Adaku *et al.*, 2022). Whether the impact of APOE on translation contributes to its effect on invasion is currently unknown. Regarding the molecular mechanism downstream of LRP8 ligation, Bhattacharjee et al. found that APOE inhibits the phosphorylation of VEGFR2, a receptor that has been shown to be essential for endothelial migration (Bhattacharjee *et al.*, 2011; Olsson *et al.*, 2006). Epistasis experiments confirmed that APOE mediates its impact on endothelial recruitment via an LRP8/VEGFR2-axis (Pencheva, 2014). Therefore, it seems plausble that this axis also mediates the differential impact of APOE variants on endothelial recruitment, warranting analogous experiments employing all three major APOE variants.

Importantly, it also remains unknown whether *APOE* genotype exerts an impact on the outcome in other cancer types. We focused our analyses on melanoma since APOE was identified as a tumor-suppressive factor in this cancer type (Pencheva *et al.*, 2012). Given our findings of an effect of *APOE* genotype on the host immune response rather than on tumor cells directly, it is plausible to hypothesize that *APOE* genotype may have similar effects in other cancer types. However, melanoma is known as a highly immunogenic tumor type. Thus, the effect of *APOE* genotype could also be masked in a context with lower baseline anti-tumor immune activity.

Since the publication of our work several publications have followed up on our findings. Houlahan et al. found that the same association between *APOE* germline variants and outcome we observed in melanoma was true in prostate cancer (Houlahan *et al.*, 2022). In thyroid cancer, it was found that the single nucleotide variant rs429358 present in *APOE4* was associated with increased risk of cancer incidence. In contrast and also consistent with our work, the rs7214 variant, present in *APOE2*, was associated with reduced incidence (Xiao and Zhao, 2022). In laryngeal squamous carcinoma, the rs429358 variant similarly was associated with a reduced incidence (Liutkeviciene *et al.*, 2022). Finally, in a computational analysis, Jerby-Arnon and Regev analyzed patient single cell RNA-sequencing data to find

that in patients with immunotherapy-resistant tumors, repression of *APOE* expression in macrophages correlated with T cell dysfunctionality (Jerby-Arnon and Regev, 2022).

Beyond the specific role of *APOE* germline variants, our study indicates that a systematic assessment of the role of germline genetics in shaping tumor progression and therapy response is warranted. The sheer number of germline variants and limited scalability for experimental testing for causal assocations represent key challenges for a systematic analysis of the impact of germline variants on tumor progression. However, the advent of pooled genetic screens employing CRISPR/Cas9 systems constitutes a promising opportunity to address this question in an efficient way. In particular, CRISPR screens employing base editing enzymes to introduce specific mutations will allow for the assessment of causal variant-phenotype associations at scale (Hanna *et al.*, 2021; Cuella-Martin *et al.*, 2021). Consistent with the notion of germline variants shaping tumor progression through the modulation of the tumor microenvironment, two recent studies systematically uncovered the association of many germline variants with altered composition of the tumor microenvironment (Pagadala *et al.*, 2022; Tian *et al.*, 2021).

However, despite these advances key challenges remain. Most importantly, several ways are conceivable of how germline variants may modulate tumor progression: potential tumor-intrinsic effects include effects on genome instability, signaling, metastatic capacity, and apoptosis resistance. In addition, variants could modulate the interplay between the TME and the tumor, including effects on immune cell function and angiogenesis. Therefore, identifying suitable model systems in which to test for variant genotype-phenotype associations is a major challenge.

3.3 Identification of common germline variation as a causal modulator of COVID-19 outcomes

The pronounced heterogeneity in clinical outcomes is a major challenge in the COVID-19 pandemic. Our study implicating *APOE* germline variants in shaping the course of SARS-CoV-2 infection is the first report of common germline variants to causally modulate outcome of COVID-19.

Many studies have asssessed the role of germline variation in COVID-19 outcome (The Severe Covid-19 GWAS Group, 2020; Asano *et al.*, 2021; Pairo-Castineira *et al.*, 2021b; COVID-19 Host Genetics Initiative *et al.*, 2021; Nakanishi *et al.*, 2021). A major characteristic distinguishing our work from these other important efforts is its reverse genetic nature: given our prior knowledge of a role of *APOE* variants in modulating immune-related processes, our analyses focused on only *APOE* rather than pursuing a genome-wide approach. Our use of a mouse model recapitulating human genetic variation allowed us to establish that the impact of *APOE* on COVID-19 is indeed causal rather than merely correlative.

A surprising finding from our study is the observation that both *APOE2* and *APOE4* mice exhibited detrimental outcomes relative to *APOE3* mice. This is in contrast to the patterns observed in Alzheimer's disease and melanoma, in which *APOE2* and *APOE4* confer opposing effects (Strittmatter *et al.*, 1993; Corder *et al.*, 1994; Ostendorf *et al.*, 2020). The stepwise pattern in Alzheimer's disease and melanoma is mirrored by the known binding affinity differences of APOE variants to several receptors, including LRP1, LDLR, and LRP8 as potential molecular mediators (Kowal *et al.*, 1990; Weisgraber *et al.*, 1982; Xian *et al.*, 2018; Chen *et al.*, 2011). Interestingly, however, the pattern we observed in COVID-19 was also evident in the association of *APOE* genotype with other phenotypes, including hypertriglyceridemia, in which both *APOE2* and *APOE4* associate with increased risks (Dallongeville *et al.*, 1992). The mechanisms underlying these different patterns across different phenotypes remain to be determined. In the case of COVID-19, we observed effects on both antiviral immunity and on viral infection. It is conceivable that differential modulation of these phenotypes by different APOE variants partially explains the non-stepwise association of *APOE* genotype with COVID-19 outcome.

Our study warrants future investigations into the molecular mechanisms mediating the impact of *APOE* genotype on antiviral immunity and viral infection in COVID-19. Regarding the impact of APOE on viral infection, it is noteworthy that APOE has been shown to directly bind to SARS-CoV-2, suggesting that APOE variants may play a role in differential viral entry by SARS-CoV-2 (Yin *et al.*, 2021). In addition, genes regulating cholesterol metabolism have been implicated in modulating SARS-CoV-2 infection (Wang *et al.*, 2021; Schneider *et al.*, 2021; Hoffmann *et al.*, 2021), providing a potential mechanistic

angle for how APOE modulates viral infection. Recently, two studies showed that APOE directly binds to the main receptor responsible for SARS-CoV-2 uptake, ACE2 (Zhang *et al.*, 2022; Chen *et al.*, 2023), although no differential binding affinities to distinct APOE variants was observed.

A major strength of our study is the comprehensive and well-powered assessment of COVID-19 in an in-vivo mouse model, including flow cytometric assessment of peripheral blood and dissociated lungs as well as single cell RNA-sequencing of several lungs. Our data reveal striking similarities between severe murine and human COVID-19, including increases in myeloid/lymphoid ratios (Lucas *et al.*, 2020); together with the recapitulation of increased COVID-19 severity mediated by sex and age, these data validate the suitability of this model for further mechanistic and longitudinal investigations of severe COVID-19 pathogenesis beyond the role of *APOE* genotype.

Our study raises several questions regarding other aspects of the COVID-19 pandemic. Importantly, over the course of the pandemic a multitude of viral strains have evolved with differing infectivity and pathogenicity (Krause *et al.*, 2021). It remains unknown whether *APOE* genotype similarly impacts the progression of different variants. It is also intriguing to speculate that *APOE* genotype may impact vaccination efficacy. Our studies were performed on a population that was mostly not-vaccinated. Given the role of *APOE* in immune modulation, it can by hypothesized that *APOE* differentially modulates response to vaccination. However, it is also conceivable that the effect of vaccination overrides the impact of *APOE* genotype on outcomes. Intruigingly, Gemmati and colleagues found that *APOE* genotype significantly associated with different levels of antibodies induced upon vaccination (Gemmati *et al.*, 2022), providing indirect support for the former hypothesis.

Finally, it remains a major open question whether *APOE* has an impact on the long-term sequelae after SARS-COV-2 infection termed long-COVID (Davis *et al.*, 2023). This is of particular interest in light of the known effects of *APOE* genotype on cognition (Gharbi-Meliani *et al.*, 2021). A major challenge in elucidating a potential role of *APOE* variants in modulating the risk of long COVID is the lack of mouse models recapitulating major features of human long COVID that would allow for employing the *APOE*-knock-in mice. In addition, it may be difficult to distinguish between a role of *APOE4* in generally altering the

threshold for cognitive impairments and a virus-specific role of *APOE* variants, something that our study of the acute sequelae of viral infection was not affected by. Interestingly, in a Finnish population of individuals with COVID-19, the *APOE4* variant was associated with cerebral microhaemorrhages and post-COVID mental fatigue, providing support for a potential link between *APOE* genotype and long COVID incidence and outcome (Kurki *et al.*, 2021). It was also shown that APOE variants differentially impact infection of neurons and astrocytes with SARS-CoV-2 as a potential mechanism underlying differential cognitive sequelae in COVID-19 (Wang *et al.*, 2021).

Several publications on the association between *APOE* genotype and COVID-19 have emerged after our study was published. Li et al. confirmed the association between the rs429358 variant and adverse COVID-19 outcomes (Li *et al.*, 2022). In a meta-analysis, Chen and colleagues also validated the clinical association between *APOE4* and detrimental outcomes in SARS-CoV-2 infection (Chen *et al.*, 2023). Of note, while our study was in press, Zhang and colleagues reported increased COVID-19 incidence and elevation of serum inflammatory markers in *APOE4* carriers (Zhang *et al.*, 2022), suggesting that just one copy of *APOE4* may be sufficient to modulate anti-viral immunity in SARS-CoV-2 infection.

3.4 Uric acid as a potential biomarker for graft versus host disease after hematopoietic stem cell transplantation

Allogeneic hematopoietic stem cell transplantation is an effective therapy for high-risk acute leukemias and other hematological diseases, but its benefits are partially offset by major therapy-related toxicities, with graft-versus-host disease constituting the chief culprit. Thus, careful balancing between positive and negative immunological consequences is required. Biomarkers predicting the risk for GVHD would be useful in individually tailoring immune-suppressive therapy post-transplantation.

In our retrospective analysis of uric acid levels in patients undergoing allo-HSCT, we found that low uric acid levels correlated with higher risk of GVHD. Our study was motivated by the known roles of uric acid as a damage associated molecular pattern (DAMP) and as

an antioxidant, two roles which we hypothesized might confer detrimental and favorable effects of uric acid on GVHD development, respectively.

Since our study was published, multiple other studies have also investigated the association between uric acid levels and GVHD incidence. A prospective multicenter study did not find significant associations between uric acid levels and GVHD in 366 allo-HSCT patients (Penack *et al.*, 2020). Ghasemi and colleagues found the same association between low uric acid levels and increased GVHD incidence in a retrospective analysis (Ghasemi *et al.*, 2020). In contrast to our and their results, in a small phase I interventional trial on 21 patients, urate oxidase-mediated depletion of uric acid resulted in a decreased rather than increased incidence of acute GVHD (Yeh *et al.*, 2014). In addition, murine preclinical data suggested a role of uric acid mediated NLRP3 inflammasome activation in triggering GVHD (Jankovic *et al.*, 2013).

Several factors potentially explain the discrepant findings between these past studies: Most importantly, uric acid levels are prone for frequent fluctuations as they are impacted by multiple variables. Amongst these, renal function and modulation by commonly administered medications in the context of allo-HSCT such as cyclosporine A and uric acid lowering medication including allopurinol represent important reasons underlying these fluctuations. In addition, the studies listed above differ in key characteristics. Most importantly, different studies measured uric acid levels at different time points. While we used uric acid levels on the day of transplantation for our calculations, the prospective study by Penack and colleagues used uric acid levels before conditioning (Penack *et al.*, 2020). Furthermore, patient characteristics differed significantly between the studies; for example, Penack and colleagues focused their study on analyzing recipients of matched sibling stem cells transplants while we also included non-sibling matched transplantations (Penack *et al.*, 2020; Ostendorf *et al.*, 2015).

In sum, the role of uric acid levels in predicting and modulating GVHD incidence remains to be conclusively determined. To this end, it will be important to harmonize the timepoints of measuring, potentially include multiple timepoints and adjust for patient and treatment characteristics.

3.5 Identification of treatment targets in myelodysplastic syndromes

In our prospective study to assess hematopoietic hierarchy and identify potential treatment targets in in myelodysplastic syndromes (MDS), we revealed aberrant expression of ALDH, CLL-1, CD44, and CD47 on specific hematopoietic progenitor cell types. Importantly, we showed that expression of CLL-1 occurs as early as on putative cancer stem cells, revealing it as a candidate target for MDS eradication.

After publication of our results, other studies revisited the phenotype of myelodysplastic hematopoietic stem cells. Dong and colleagues confirmed the aberrant expression of CD47 on $CD34^{+}CD38^{-}$ cells in MDS, which are enriched for MDS stem cells (Dong *et al.*, 2022). In a study employing mass cytometry, increased CD44 was confirmed on MDS stem cells (Bachas *et al.*, 2023).

In addition to these diagnostic studies, progress has been made in advancing therapeutic targeting of some of the markers described in our study and those of other groups: a recent clinical phase 1 study included individuals with high-risk MDS for the assessment of a CD47-directed antibody (Zeidan *et al.*, 2022). In addition, targeting CLL1 has entered clinical trials for AML, either by employing a CLL1/CD3-bispecific antibody or by administration of CLL1-directed CAR-T cells (Ma *et al.*, 2019).

Most of the aberrant marker expression we observed was only evident in patients with MDS with excess blasts. While identifying novel treatment approaches is particularly pressing in this high-risk group, identifying aberrant marker expression in patients with low risk MDS with non-elevated blast counts would be highly desirable to aid in establishing the diagnosis (Mufti *et al.*, 2018). Technologies that have entered mainstream use since the publication of our study have already proven highly useful in more comprehensively phenotyping the hematopoietic landscape in these patients, including single cell RNA-sequencing (scRNA-seq) and single cell proteomics. For example, recent work has employed mass cytometry and scRNA-seq to identify several pathways as potential treatment targets in MDS stem cells (Stevens *et al.*, 2018; Ganan-Gomez *et al.*, 2022).

Despite these technological advances no single target on MDSC cancer stem cells has been identified so far that would allow for universal MDS therapy (Zhan and Park, 2021). Among other promising targets are CD99 as reported by Chung and colleagues (Chung *et al.*, 2017). In high-risk MDS, CD123 may be a viable treatment target (Ganan-Gomez *et al.*, 2022). Whether targeting any of the markers discussed will prove sufficient to eradicate MDS remains to be determined in pre-clinical and clinical studies (Zhan and Park, 2021).

4 Summary

Precision medicine refers to the tailoring of medical management to the individual patient. Despite the advent of increasingly high-dimensional diagnostic tools and the enormous expansion of our therapeutic arsenal, precision medicine remains a promise rather than reality for most patients. A major factor responsible for making the realization of precision medicine challenging is the lack of a mechanistic understanding of how given biological variables impact the course of a disease.

This work describes five studies which tackle different facets of precision medicine: in the first study, we report preclinical and clinical data of a novel therapeutic approach to harness a patient's immune response to cancer by targeting suppressive immune cells in the tumor microenvironment (TME). The pharmacologic activation of Liver-X-receptor transcription factors led to depletion of immunosuppressive myeloid-derived suppressor cells in an APOE-dependent manner. The resulting unleashing of anti-tumor T cell immunity represents a novel myeloid cell-targeting approach for cancer immunotherapy.

In studies two and three, we established *APOE* genotype as a potential biomarker to predict outcome in melanoma and COVID-19. Using mice bearing distinct human *APOE* variants, we found that the Alzheimer's disease predisposing *APOE4* variant enhances anti-melanoma immunity, while *APOE2* dampens it. Interestingly, in COVID-19, we found that both *APOE4* and *APOE2* causally mediate detrimental outcomes. These discrepant phenotypic patterns can likely be explained by the pleiotropic roles of APOE, which we determined to include modulation of antiviral immunity and viral infection in the case of SARS-CoV-2 infection.

In the fourth study, we investigated the potential role of uric acid levels as a biomarker for acute graft versus host disease. Finally, in the fifth study we dissected cancer stem cells in myelodysplastic syndromes to identify targets for potential novel therapeutic approaches.

We identified CLL1 to be expressed on MDS cancer stem cells, validating its potential as a target for future therapies.

In sum, the work presented herein spans a precision medicine spectrum from disease prediction over target identification to therapeutic assessment of new targeted therapies. It contributes to addressing the need to elucidate the mechanisms underlying differential disease courses and treatment responses across patients to unlock precision medicine for all patients.

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Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
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- mir die geltende Habilitationsordnung bekannt ist.

  Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

Datum

Unterschrift