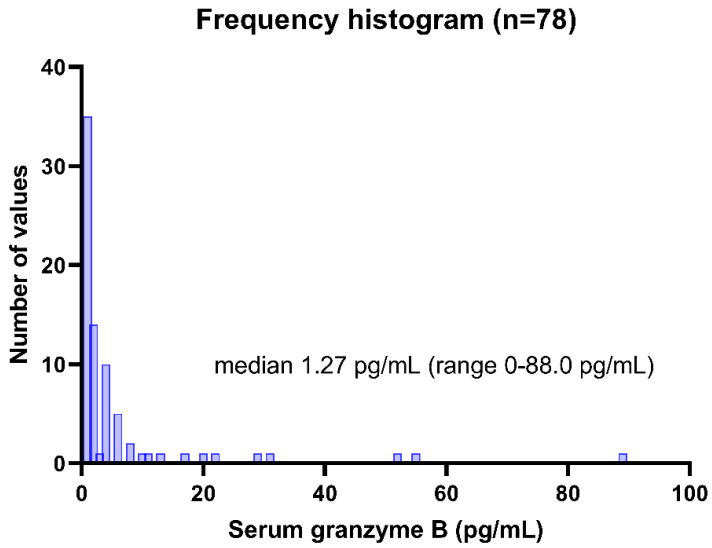
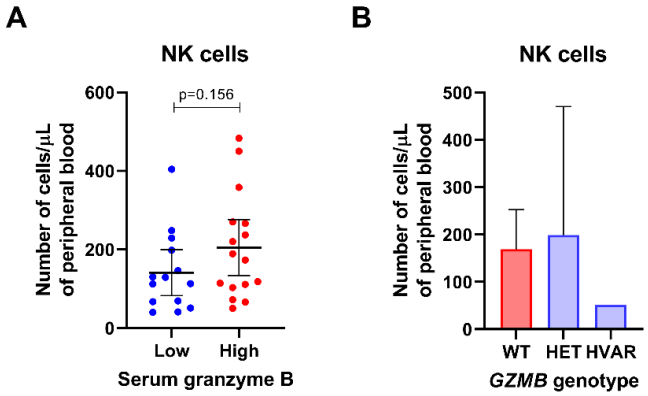
Supplementary data

Supplementary Figure 1: Frequency histogram



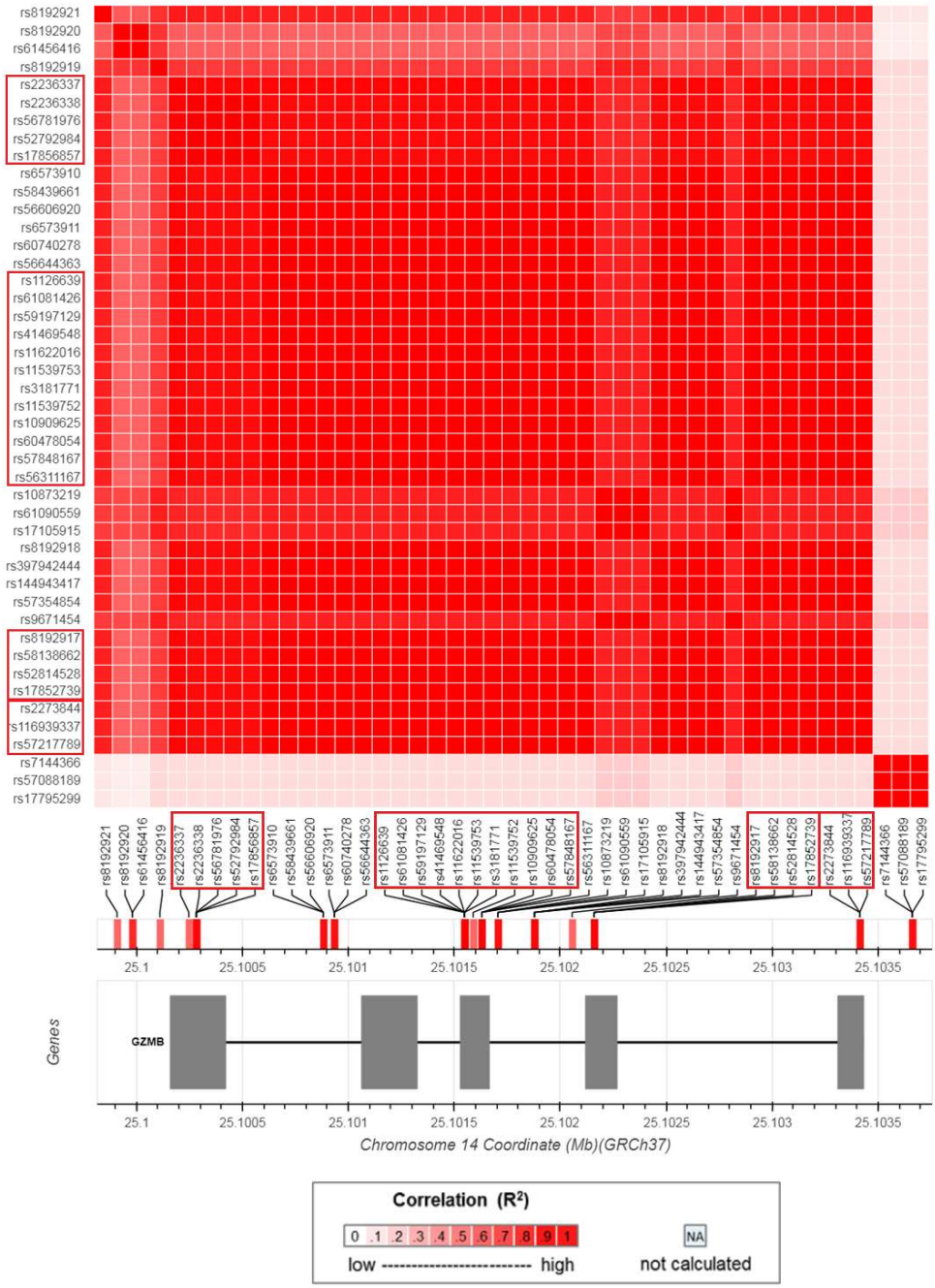
Suppl. figure 1: Frequency histogram of granzyme B measurements in serum. In total, baseline measurements were performed in 78 stage IV NSCLC patients. Almost half of the patients had undetectable levels of granzyme B in serum.

Supplementary Figure 2: Lack of association between serum granzyme B or GZMB genotypes and NK cell numbers



Suppl. figure 2: Association are shown between A) serum granzyme B levels or B) GZMB *genotype and the number of CD56+ NK cells in blood. Abbreviations: wild type (WT), heterozygous variant (HET) and homozygous variant (HVAR).*

Supplementary Figure 3: LD statistics for all common *GZMB* SNPs



Suppl. figure 3: Heatmap matrix of pairwise linkage disequilibrium statistics of common SNPs of GZMB (MAF > 15%; dbSNP database). Exon germline variants (rs-numbers) are marked with the red boxes. Note that all common germline variants present in GZMB exones are in strong linkage disequilibrium, defining two predominant GZMB isoforms in the European population (~70% common GZMB allele). Data derived from publicly available reference haplotypes are used from the 1000 Genomes Project. The R squared as a measure of linkage disequilibrium are shown, red indicates high correlation. Abbreviations: R squared (R^2).

Supplementary table 1: Association of SNPs with AIDs

Gene	Rs-number	Disease	Effect	Key references
HLA-A	rs60131261	Vitiligo	Higher susceptibility (OR 1.53) for AID and associated with elevated expression of HLA-A	Jin, 2016[15]
GZMB	rs8192917	Vitiligo	Higher susceptibility (OR 1.23/1.39) for AID	Jin, 2016[15] Xu, 2018[16]
IL10	rs3024493	SLE, T1D, CD, UC, BeD	Higher susceptibility (OR 1.26) for AIDs	Ramos, 2011[17]
IL2RA	rs2104286	RA, MS	Lower susceptibility (OR 0.81) for RA, lower susceptibility (OR 0.84) for MS	Kurreeman, 2009[18] Wang, 2018[19]
IFNG	rs2430561, rs2069705, rs2069718	SLE	Higher susceptibility (OR 1.75/2.42) for AID	Lee, 2016[20] Kim, 2010[21]
PDCD1	rs2227981, rs10204525, rs2227982	AS	Higher susceptibility for AID	Chen, 2016[22] Liu, 2011[23]
PTPN11	rs2301756	UC	Higher susceptibility (OR 1.81) for AID	Narumi, 2009[24]
ZAP70	rs13420683	CD	Lower susceptibility (OR 0.44) for AID	Bouzid, 2013[25]

Suppl. table 1: Germline variations of genes that are implicated in the etiopathogenesis of autoimmune diseases (AIDs). These variants are indicated by gene and rs-number. Abbreviations: Type 1 diabetes (T1D), Crohn's disease (CD), ulcerative colitis (UC), alkylosing spondyloditis (AS), Behcet's disease (BeD), rheumatoid arthritis (RA), multiple sclerosis (MS), systemic lupus erythematosus (SLE).

Supplementary table 2: Investigated single-nucleotide polymorphisms

Protein	Gene	rs-number	Variant	WT	HET	HVAR	Undet.	MAF	HWE (χ^2)
Granzyme B	*GZMB*	rs8192917	c.128T>C	205	104	13	0	20%	<0.01
HLA-A	*HLA-A*	rs60131261	delTTTA	178	116	28	0	27%	2.05
IL-10	*IL10*	rs3024493	c.387+284C>A	231	81	10	0	16%	0.77
CD25	*IL2RA*	rs2104286	c.64+5006T>C	180	118	22	2	25%	0.2
IFN-γ	*IFNG*	rs2430561	c.874T>A	105	153	63	1	43%	0.29
		rs2069705	−1616T>C	145	135	42	0	34%	1.4
		rs2069718	367–895C>T	112	148	60	2	42%	0.8
PD-1	*PDCD1*	rs2227981	804C>T	94	154	73	1	47%	0.42
		rs10204525	889G>A	271	47	4	0	9%	1.39
		rs2227982	644C>T	320	2	0	0	0.3%	-
SHP-2	*PTPN11*	rs2301756	333–223A>G	259	60	3	0	10%	0.05
ZAP-70	*ZAP70*	rs13420683	−21–4127C>A	177	106	33	6	27%	7.42*

*Suppl. table 2: Overview of investigated SNPs. *If χ^2>3.84 (p-value <0.05) then SNP is not consistent with HWE.* ZAP70 -32-4127C>A (rs13420683) was not consistent with HWE (χ2 7.42; p < 0.01) but remained in the analysis after excluding the likelihood of a type I error. As PDCD1 644C>T (rs2227982) had a MAF of 0.3% (<5%) in the study cohort, it was excluded from further analyses. All other SNPs were considered suitable for analysis. *Abbreviations: wild-type (WT), heterozygote variant (HET), homozygote variant (HVAR), minor allele frequency (MAF), Hardy-Weinberg equilibrium (HWE).*

Supplementary table 3: Internal validation

Multivariate analysis (BOR)					
Factor	**Comparison**	**OR (95%CI)**	**P-value**	**Bias**	**Bias-corrected 95%CI**
GZMB c.128T>C	CC + CT vs. TT	1.019-2.509	0.041	0.006	1.052-2.548
Primary tumor	Other vs. adeno	0.410-0.981	0.041	-0.005	0.425-1.009
IL-10 c.387+284C>A	AA vs. AA +AC	0.010-5.420	0.367	-0.128	0.033-0.908
Primary tumor	Other vs. adeno	0.398-0.946	0.027	-0.006	0.405-0.979
ZAP70 -21-4127C>A	AA vs. CC+AC	0.236-1.019	0.056	0.005	0.246-1.014
Primary tumor	Other vs. adeno	0.395-0.953	0.030	-0.006	0.398-0.987
Multivariate analysis (PFS)					
Factor	**Comparison**	**HR (95%CI)**	**P-value**	**Bias**	**Bias-corrected 95%CI**
GZMB c.128T>C	CC+CT vs. TT	1.022-1.867	0.036	0.003	1.027-1.879
WHO performance	0 vs. ≥1	0.418-0.898	0.012	<-0.001	0.408-0.876
Sex	Male vs. female	0.943-1.771	0.111	<0.001	0.943-1.780

Suppl. table 3: Bootstrap results of the association between SNPs and clinical outcome. Bias measure, bias-corrected 95%CI and p-value of the multivariate analysis are shown in the table for each factor. SNPs associated with BOR, PFS or OS (p<0.1; multivariate analysis) were included. Remaining SNPs and patient factors are not shown. Abbreviations: hazard ratio (HR), odds ratio (OR), 95% confidence interval (95%CI).*

Supplementary table 4: Association of PD-L1, TMB and serum granzyme B with PFS

PFS			**Univariate**			**Multivariate**		
		n	**HR**	**95%CI**	**p-value**	**HR**	**95%CI**	**p-value**
Expression of PD-L1 in tumor	<50 vs. ≥50%	26	4.30	0.93-19.77	0.06	4.04	0.36-45.31	0.26
Tumor mutational burden	<10 vs. ≥10 mut/Mb	22	7.31	1.54-34.67	0.01*	3.42	0.60-19.53	0.17
Serum granzyme B	<1.27 vs. ≥1.27 pg/mL	26	2.34	0.93-5.93	0.07	1.53	0.52-4.51	0.44

*Suppl. table 4: Univariate and multivariate analysis of the association between expression of PD-L1 in tumor, tumor mutational burden and serum granzyme B levels with progression-free survival (PFS). Biomarkers associated with PFS (p-value<0.1) were included in the multivariate analysis. COX regression analysis was performed on a subset of patients from cohort 1, of whom baseline parameters and clinical outcome was available. Significance is marked by *. Abbreviations: hazard ratio (HR), 95% confidence interval (95%CI).*

Supplementary table 5: Association of PD-L1, TMB and serum granzyme B with OS

OS			Univariate			Multivariate		
		n	**HR**	**95%CI**	**p-value**	**HR**	**95%CI**	**p-value**
Expression of PD-L1 in tumor	<50 vs. ≥50%	26	4.04	0.90-18.15	0.07	3.22	0.28-36.50	0.35
Tumor mutational burden	<10 vs. ≥10 mut/Mb	22	3.42	0.93-12.66	0.07	1.65	0.36-7.54	0.52
Serum granzyme B	<1.27 vs. ≥1.27 pg/mL	26	2.95	1.09-7.93	0.03*	2.25	0.73-6.91	0.16

*Suppl. table 5: Univariate and multivariate analysis of the association between expression of PD-L1 in tumor, tumor mutational burden and serum granzyme B levels with overall survival (OS). Biomarkers associated with OS (p-value<0.1) were included in the multivariate analysis. COX regression analysis was performed on a subset of patients from cohort 1, of whom baseline parameters and clinical outcome was available. Significance is marked by *. Abbreviations: hazard ratio (HR), 95% confidence interval (95%CI).*

Supplementary table 6: Correlation matrix of PD-L1, TMB and granzyme B

		PD-L1 expression	Tumor mutational burden
Tumor mutational burden	Pearson Correlation	0,13	
	p-value (2-tailed)	0,56	
	n	22	
Granzyme B	Pearson Correlation	-0,20	-0,18
	p-value (2-tailed)	0,33	0,42
	n	26	22

Suppl. table 6: Correlation matrix showing the Pearson correlation test outcomes of tumor mutational burden, granzyme B levels and PD-L1 expression in tumor tissue obtained prior to start of PD-1 ICIs in a subset of patients from cohort 1. Abbreviations: number of patients (n).