Integrated whole genome and transcriptome analysis identified a therapeutic minor histocompatibility antigen encoded by an alternative *ITGB2* transcript

Margot Pont

Department of Hematology
Leiden University Medical Center
Leiden, the Netherlands

SITC Poster #297

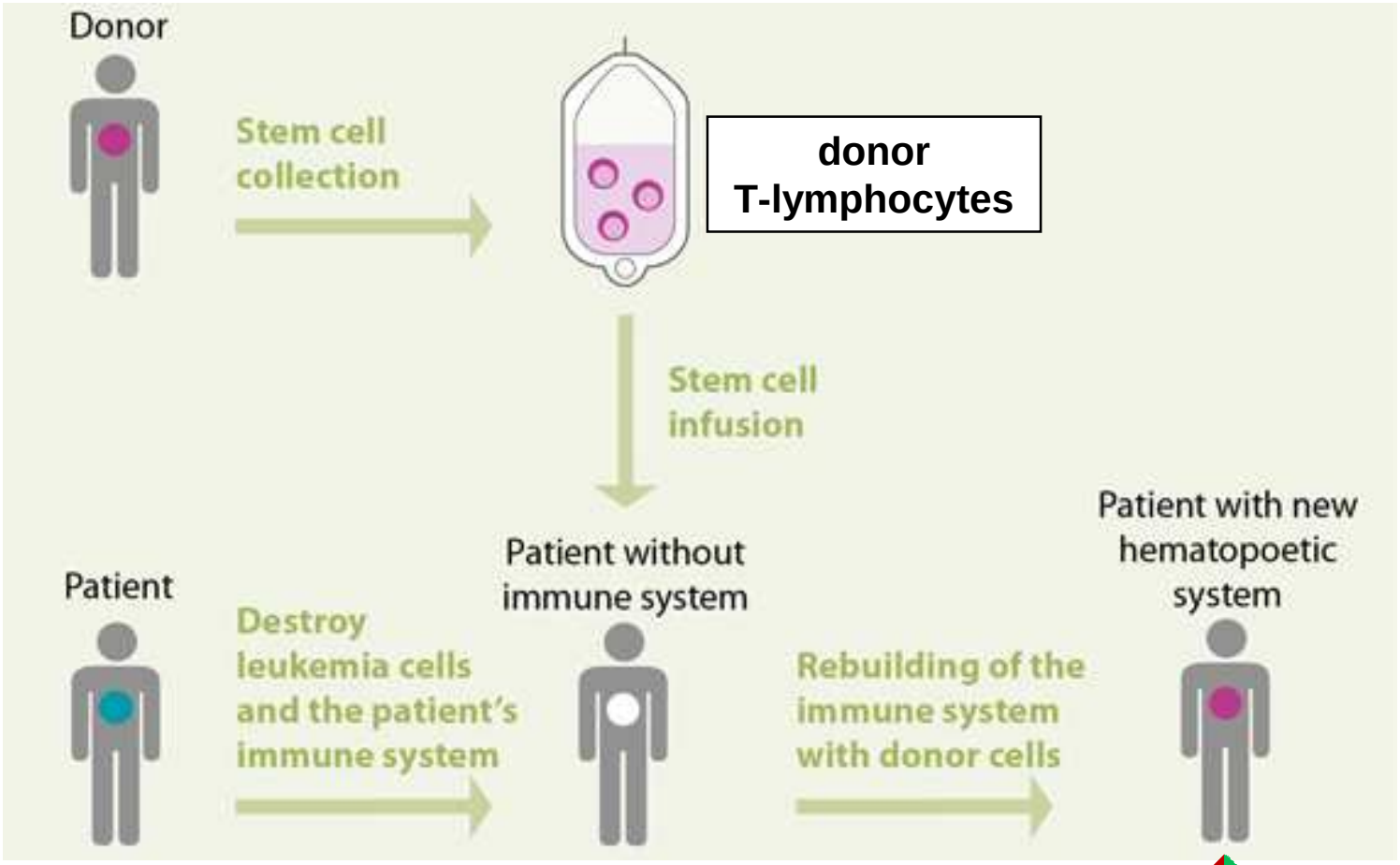
Presenter Disclosure Information

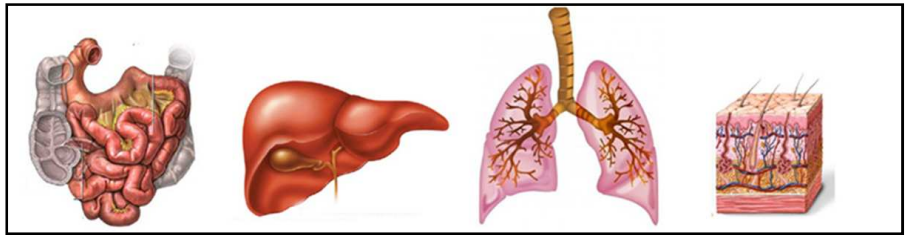
Margot Pont

The following relationships exist related to this presentation:

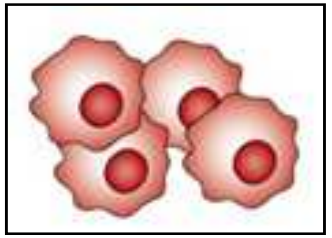
No Relationships to Disclose

Allogeneic Hematopoietic Stem Cell Transplantation (alloSCT)



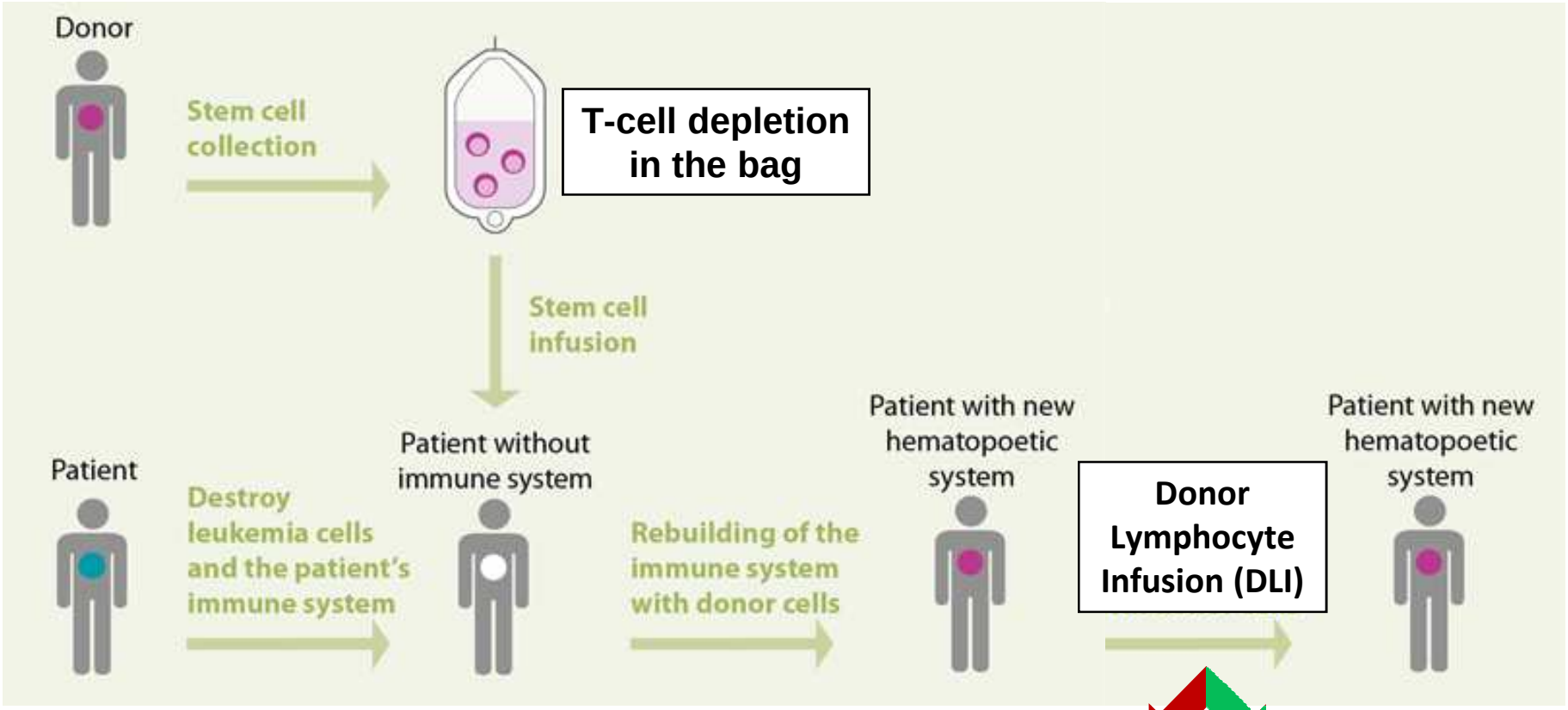


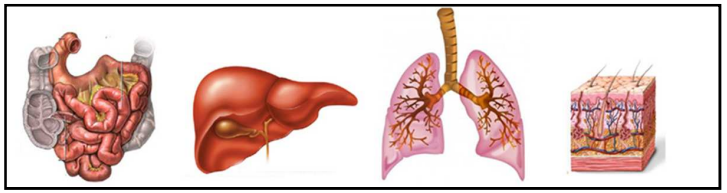
undesired side effects against healthy tissues



desired anti-tumor immunity

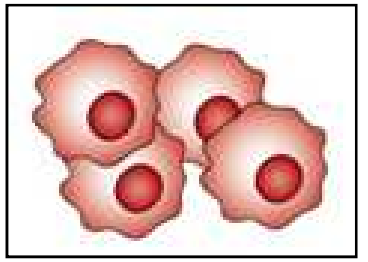
T-cell depleted allogeneic stem cell transplantation and DLI





undesired side effects against healthy tissues

Graft-versus-Host Disease (GvHD)



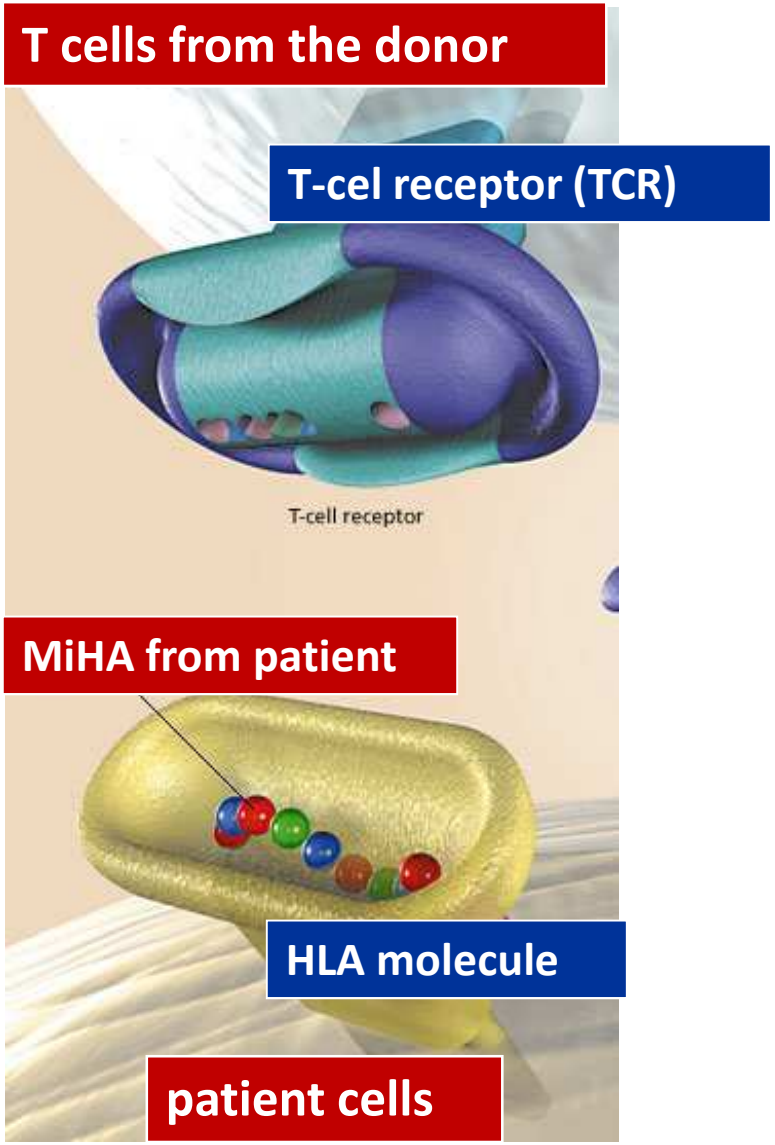
desired anti-tumor immunity

Graft-versus-Leukemia (GvL) reactivity

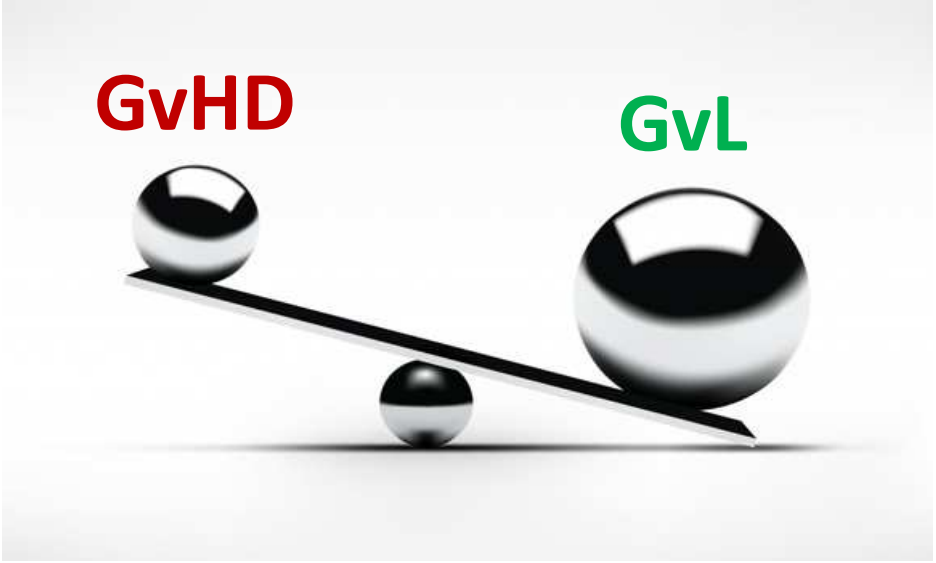
Donor T-cells recognize polymorphic antigens (MiHA) presented by shared HLA after alloSCT

In HLA-matched alloSCT for hematological malignancies, donor T-cells can cause:

- beneficial anti-tumor effect (**GvL**) when polymorphic antigens (MiHA) are expressed on the **malignant cells** of the patient
- **GvHD** when MiHA are expressed on **healthy non-hematopoietic tissues** of the patient



Minor histocompatibility antigens for immunotherapy

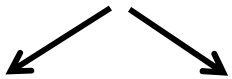


Minor histocompatibility antigens with restricted expression on cells of the hematopoietic system can be used as tumor antigens

→ More hematopoiesis-restricted MiHA need to be identified

Isolation and screening of allo-reactive T-cells

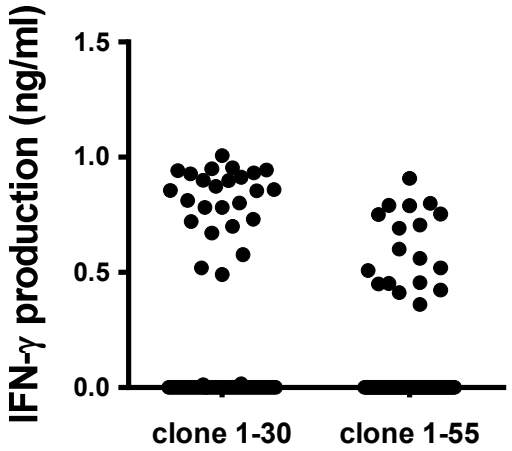
- Selection of patients with anti-tumor response after alloSCT
- Isolation of patient-specific T cells based on expression of CD137 after stimulation with patient cells
- Clonal expansion
- Screening for allo-reactivity:
  - Patient EBV-B cells
  - Donor EBV-B cells
  - Patient skin-derived fibroblasts
  - Patient skin-derived fibroblasts + IFN-γ



GvHD GvL →identify T-cell epitopes for therapeutic applications

Whole Genome Association scanning for identification of polymorphic antigens

- EBV-B cell lines from 80 individuals (HLA-A*02:01 & B*07:02)
- 1M SNPs measured by Human 1M Illumina array
- T cell reactivity against 80 EBV-B cell lines determined
- EBV-B cell lines are divided in positive and negative groups
- Association between T cell recognition and each SNP is tested



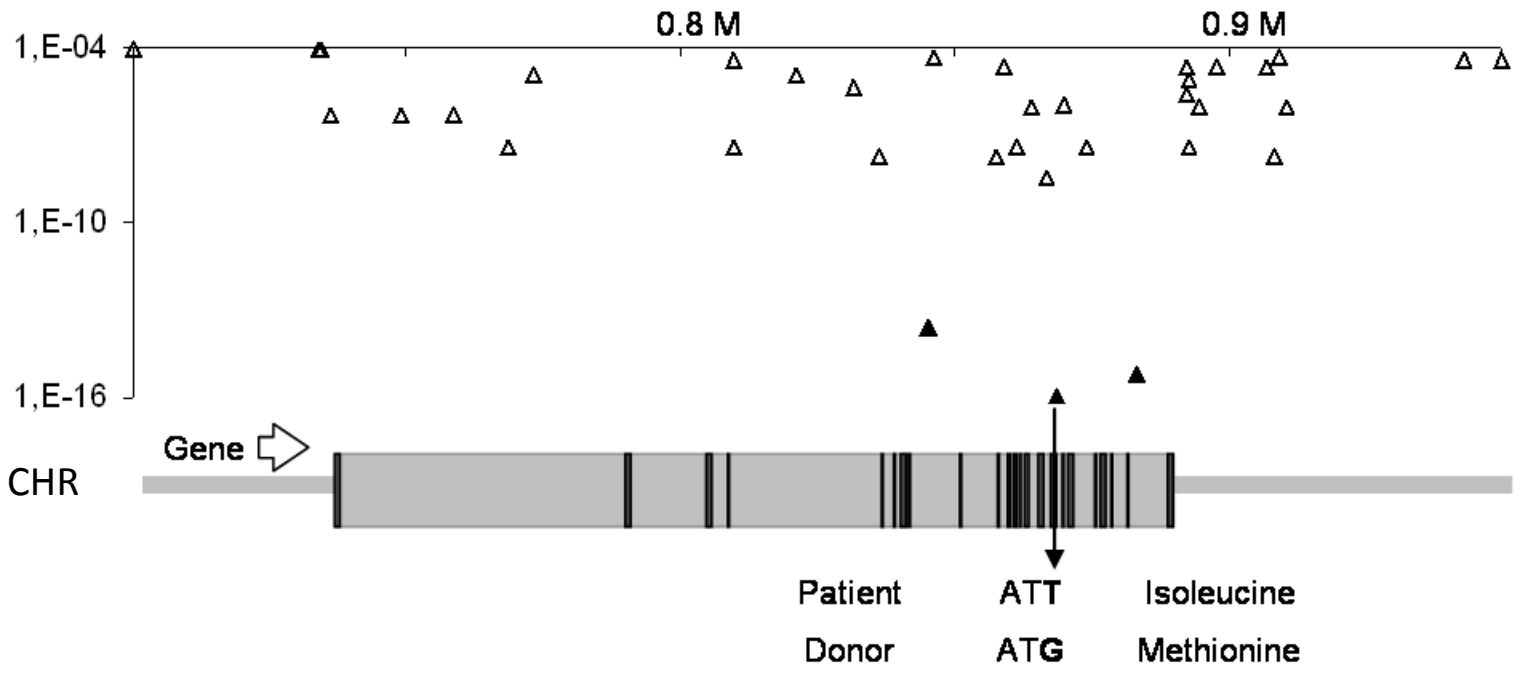
van Bergen et al, Cancer Res, 2010

Identification of polymorphic antigens using WGAs

Phenotype based on recognition of EBV-B cells

↓

Test for association between phenotype and SNP genotypes



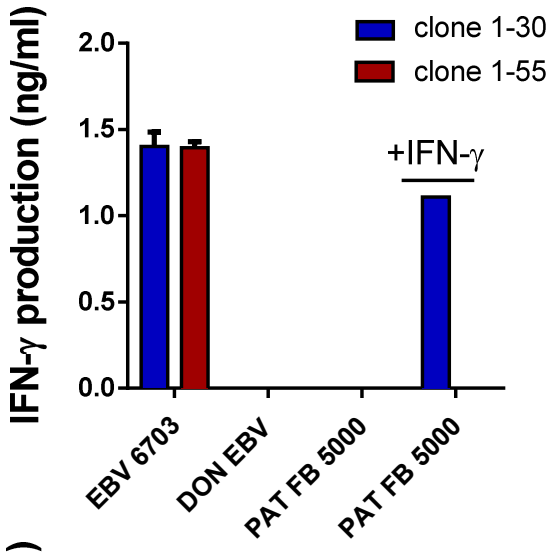
Minor histocompatibility antigens encoded by alternative transcripts

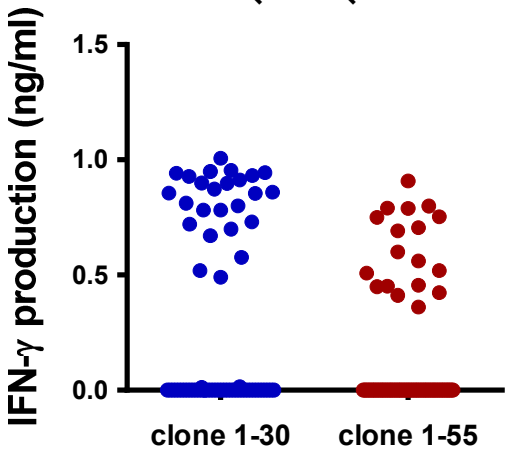
- Minor histocompatibility antigens encoded by alternative transcripts are a relevant category of T-cell epitopes

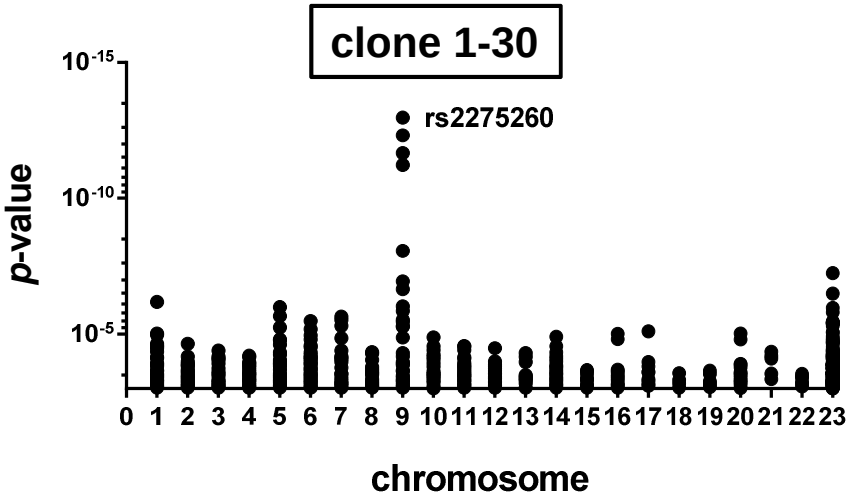
- For ~30% of antigens: associating SNPs found by WGAs, but primary gene transcript does not contain polymorphisms between patient and donor

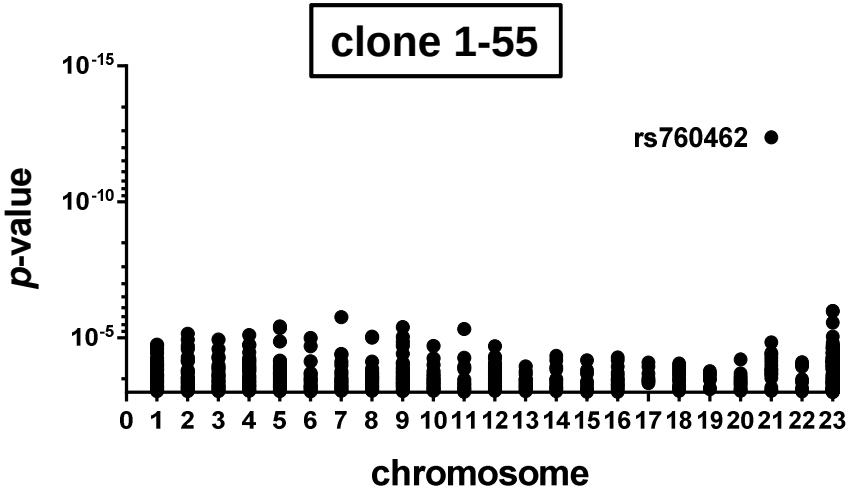
→How can we identify MiHA encoded by alternative transcripts?

Isolation of T-cell clones 1-30 and 1-55 from patient 6940



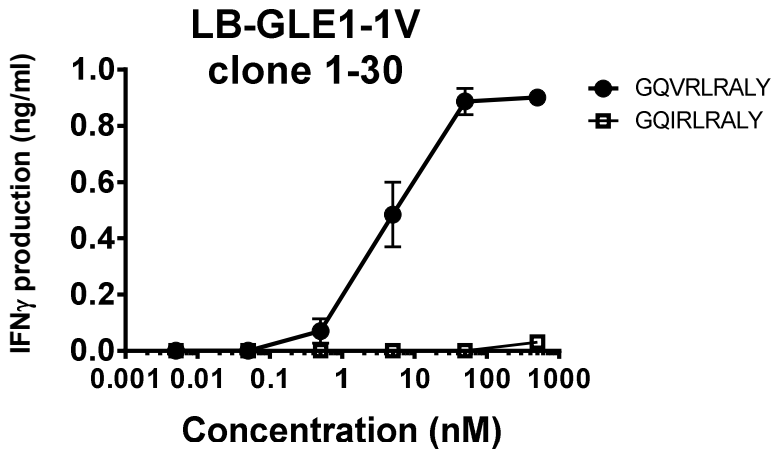






T-cell clones 1-30 and 1-55 recognize new minor histocompatibility antigens in HLA-B*15:01

Minor histocompatibility antigens in patient 6940



- **LB-GLE1-1V was directly encoded by rs2275260, located in an exon**
- **Clone 1-55 associated with rs760462, located in an intron of *ITGB2***
  - no SNP disparity was found between patient and donor in the primary gene transcript

RNA sequence data were analyzed to investigate whether alternative transcripts are derived from the *ITGB2* gene

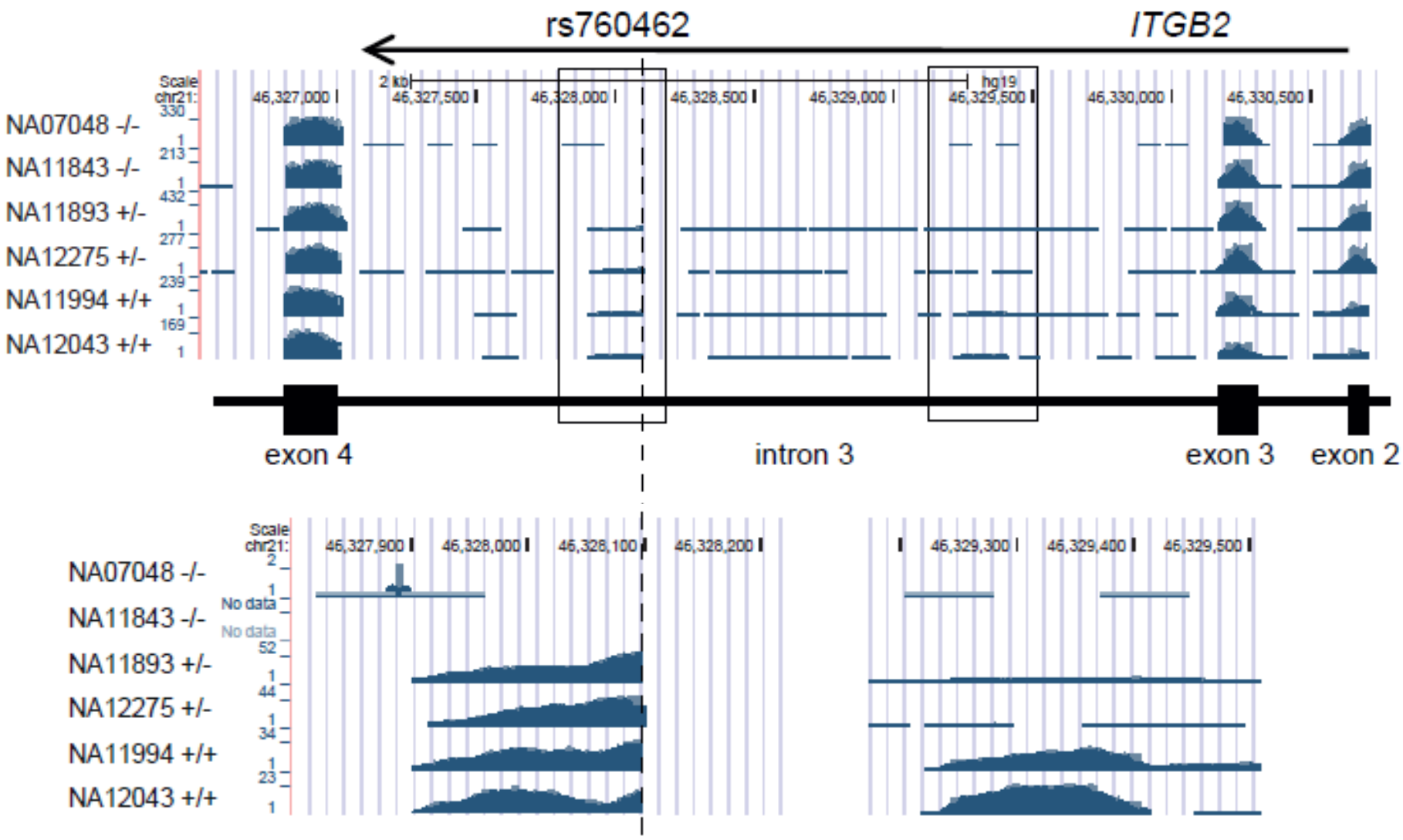
RNA sequence analysis to identify alternative transcripts

GEUVADIS project

- 462 EBV-B cell lines from 1000 Genome Project
- RNA seq with Illumina HiSeq2000
  - Paired end 75 bp mRNA
- Selection of individuals based on associating SNP genotype as identified by WGAs (+/+, +/-, -/-)

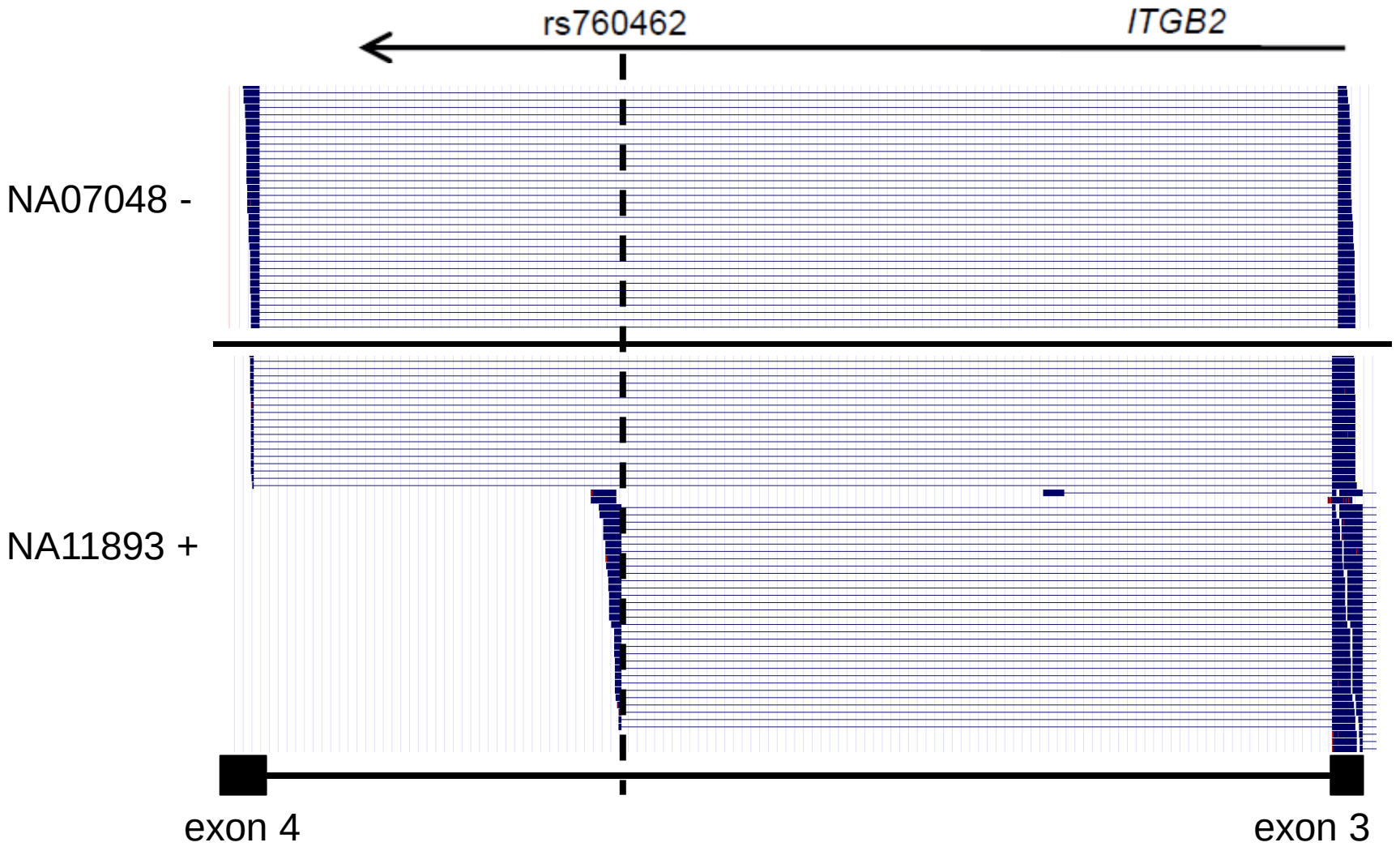
Lappalainen et al, Nature, Sept 2013; ‘t Hoen et al, Nature Biotechnology, Nov 2013

RNA sequence analysis to identify alternative transcripts



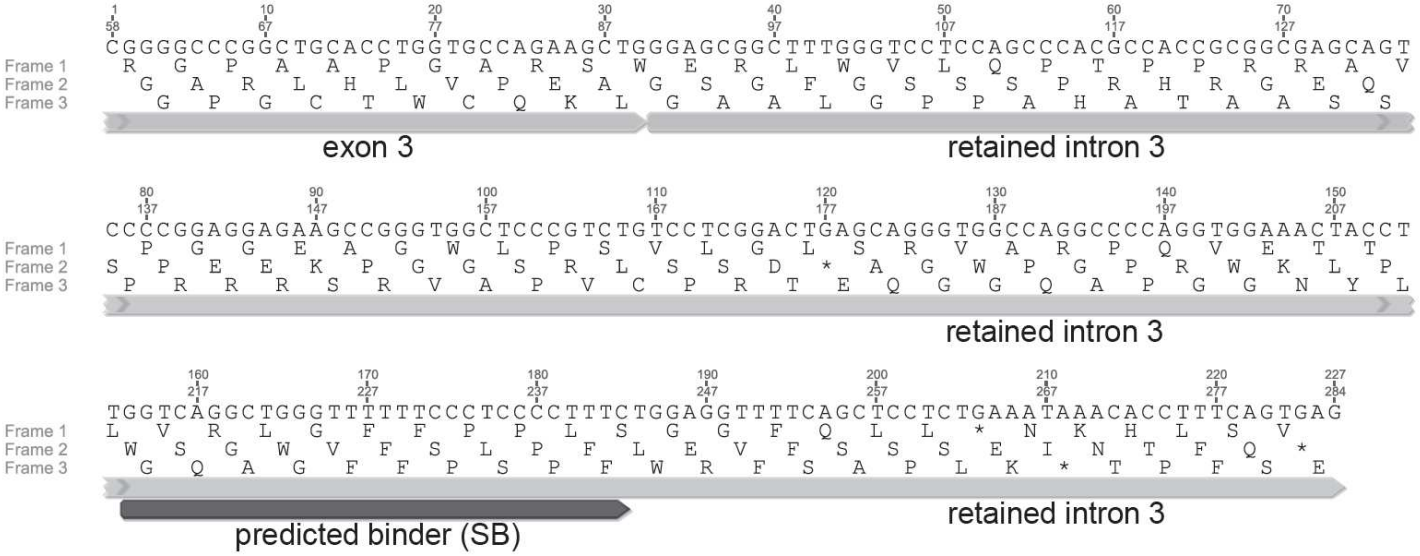
The associating SNP as identified by WGAs is associated with a transcriptionally active region in intron 3

RNA sequence analysis to identify alternative transcripts



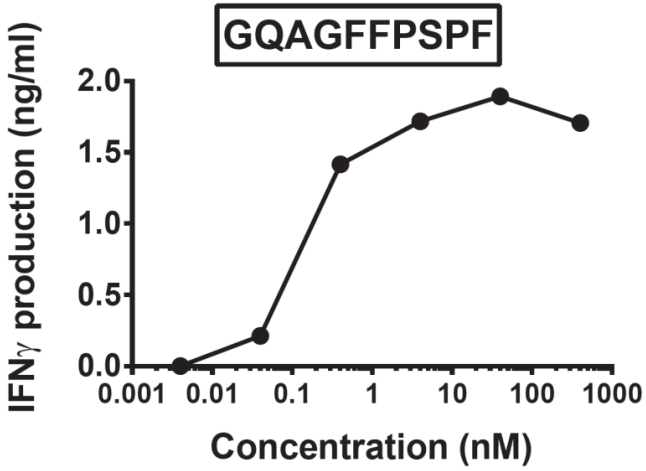
An alternative transcript is expressed in which exon 3 is fused to intron 3 sequences downstream from the associating SNP

RNA sequence analysis to identify alternative transcripts



Predicted peptides for ITGB2

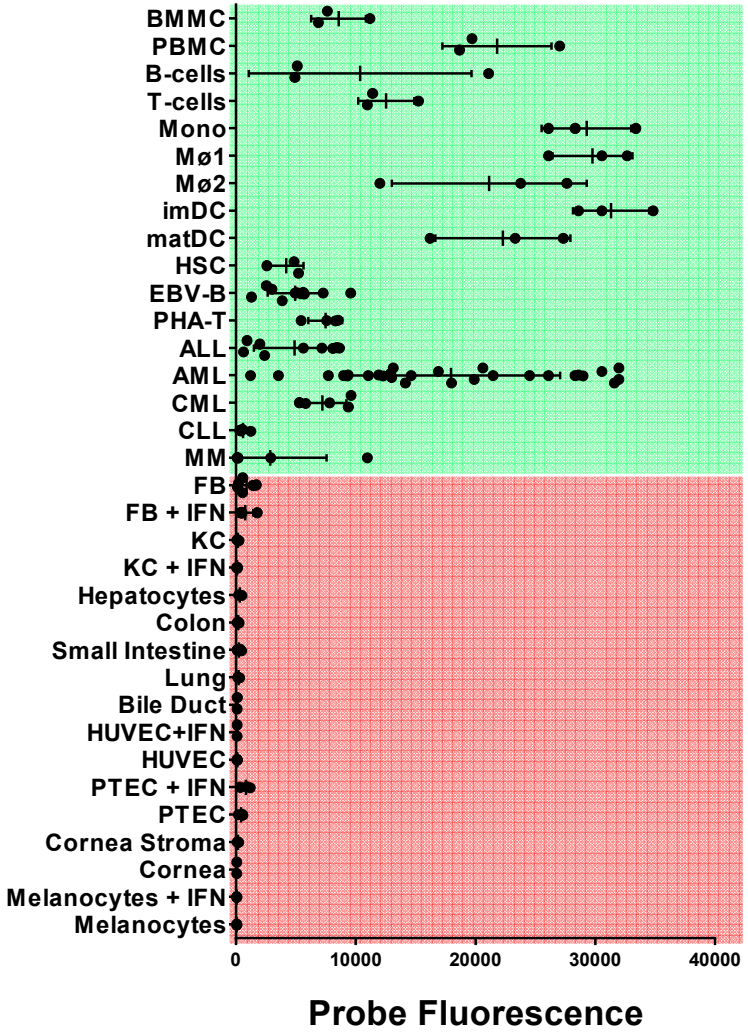
frame	length (aa)	peptide	logscore	affinity(nM)	Bind Level
1	11	LGFFPPLSGGF	0.477	286	WB
2	9	GQAPGGNYL	0.545	136	WB
2	10	GQAGFFPSPF	0.735	17	SB
2	11	EQGGQAPGGNY	0.435	451	WB
2	11	LGQAGFFPSPF	0.613	65	WB
3	9	LVPEAGSGF	0.565	110	WB
3	10	HLVPEAGSGF	0.544	138	WB



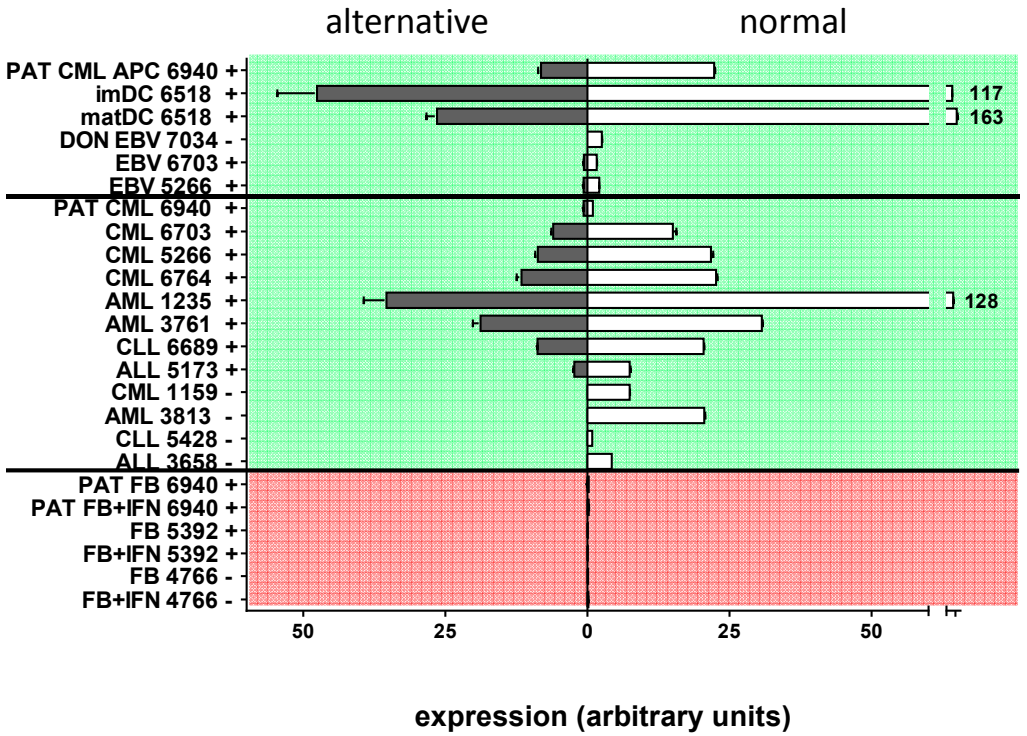
RNA sequence analysis enabled identification of LB-ITGB2-1 encoded by an alternative gene transcript

Hematopoiesis-restricted expression of *ITGB2*

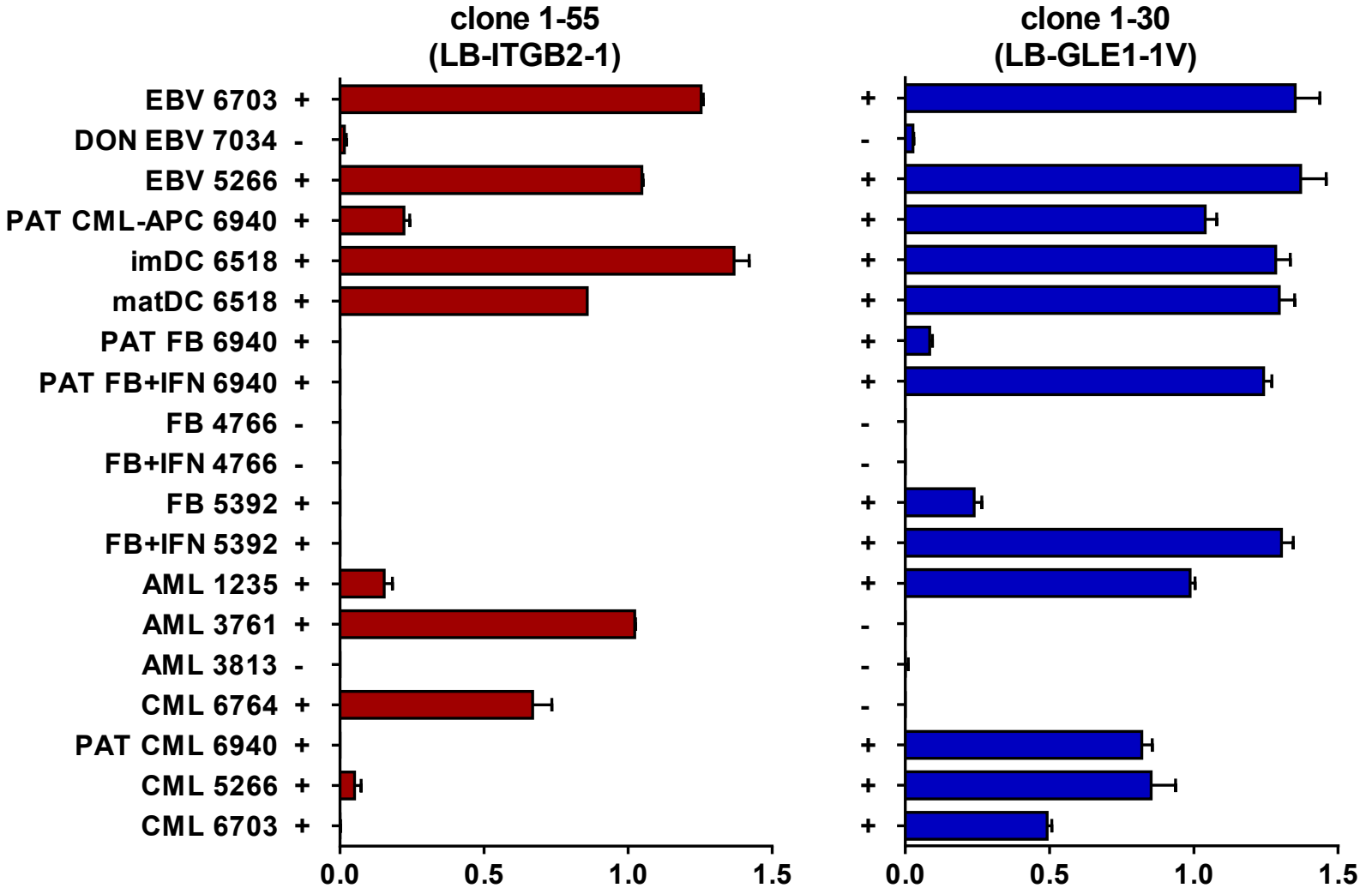
Microarray gene expression



q-PCR analysis



Hematopoiesis-restricted expression of *ITGB2*



LB-ITGB2-1 is a new hematopoiesis-restricted minor histocompatibility antigen

Conclusions and take home message

- RNA-seq analysis resulted in the identification of LB-ITGB2-1, which is a polymorphic antigen (MiHA) encoded by an alternative gene transcript

- LB-ITGB2-1 is selectively expressed on hematopoietic cells as confirmed by recognition, microarray gene expression and q-PCR analysis
- LB-ITGB2-1 can mediate specific lysis of primary leukemic samples of myeloid origin, supporting its value for T cell therapy to augment anti-tumor reactivity after alloSCT without side effects.

→ Our data show that WGAs followed by RNA-seq analysis enables identification of polymorphic antigens (MiHA) encoded by alternative transcripts

→ RNA-seq analysis provides insight in transcriptional activity and architecture, and may be of value for identification of non-polymorphic or mutated antigens encoded by alternative transcripts (solid tumors / autoimmunity)

Integrated whole genome and transcriptome analysis identified a therapeutic minor histocompatibility antigen encoded by an alternative *ITGB2* transcript

Dept. of Hematology (LUMC)
Kees van Bergen
Edith van der Meijden
Dyantha van der Lee
Willy Honders
Michel Kester
Marieke Griffioen
Fred Falkenburg

Dept. of Human Genetics (LUMC)
Peter-Bram ‘t Hoen
Martijn Vermaat

Dept. of Biostatistics (LUMC)
Szymon Kielbasa

SITC Poster #297

SITC 2015

DUTCH CANCER SOCIETY

DTIWP