Supplementary figures

Supp. Figure 1. Real time killing of NLR-labelled A375 by 10 nM HLA-A2 WT1-TCB in the presence of escalating concentrations of dasatinib. NLR-labelled A375 target cells were co-cultured with PBMCs and HLA-A2 WT1-TCB in medium supplemented with dasatinib, at E:T=10:1. Killing was followed by Incucyte (1 scan every 3 hrs, zoom 10x, phase and red, 400 ms acquisition time). The percentages of killing were measured by normalizing the total red areas with values at t = 0 hr and the control wells containing target cells, PBMCs and dasatinib for each time points. Mean of n=3 donors +/- SEM with * $p \leq 0.05$, ** $p \leq 0.01$ by 1 way ANOVA (Friedman test).

Supp. Figure 2. Dasatinib does not affect T cell viability and SKM-1 and MKN45 tumor cell growth **(A)** Effect of 100 nM dasatinib on NLR-labelled SKM-1 tumor cells growth. (**B**) Effect of 100 nM dasatinib on NLR-labelled A375 tumor cell growth. (**C**) Effect of 100 nM dasatinib on NLR-labelled MKN45 tumor cell growth. Tumor cell growth was followed by Incucyte (1 scan every 3 hrs, zoom 10x, phase and red, 400 ms acquisition time) and the total red area was normalized by the baseline at t=0 hr. Mean of n=2 donor +/- SD * $p \leq 0.05$, ** $p \leq 0.01$ by paired t test. (**D**) Effect of escalating concentrations of dasatinib on the viability of CFSE-labelled SKM-1 cells. At 24 hrs, cell viability was measured by flow cytometry using a Live/Dead stain. Mean of n=2 donors. (**E**) Effect of escalating concentrations of dasatinib on CD4+ and CD8+ T cell viability. At 24 hrs, the viability of CD4+ and CD8+ T cells was measured by flow cytometry using a Live/Dead stain and normalized by that in the absence of dasatinib (DMSO control), mean of n=3 donors.

Supp. Figure 3. Effect of dasatinib on WT1 target expression by SKM-1 tumor cells. SKM-1 cells were incubated in medium supplemented with 6.25, 25 or 100 nM dasatinib for 24 hrs. At 24 hrs, a Jurkat NFAT reporter cell assay (E:T= 3:1) was conducted in the presence of escalating concentrations of HLA-A2 WT-TCB and the Luminescence was acquired with a Perkin Elmer plate reader. Induction of the luciferase reporter gene was similar irrespective of SKM-1 cell pre-treatment with dasatinib, showing that WT1 expression was not modified.

Leclercq G, *et al. J Immunother Cancer* 2021; 9:e002582. doi: 10.1136/jitc-2021-002582

Supp. Figure 4. (A, B) Effect of escalating concentrations of dasatinib on CD4+ and CD8+ T cells proliferation. CFSE-labelled SKM-1 tumor cells were co-cultured with CTV-labelled PBMCs and HLA-A2 WT1-TCB, at E:T=5:1. To assess the effect of dasatinib on T cell proliferation, the dilution of the CTV dye in CD4+ and CD8+ T cells was measured by flow cytometry at t=72 hrs. Histogram plots are shown for 2 donors additional to the one shown on Figure 1. (**C**) Dasatinib rapidly stops CD4+ and CD8+ T cell proliferation. CTV-labelled PBMCs were co-cultured with SKM-1 tumor cells (E:T=1:1) and HLA-A2 WT1-TCB. Dasatinib was added after 24 hrs of activation. To assess the effect of dasatinib on T cell proliferation, the dilution of the CTV dye in CD4+ and CD8+ T cells was measured by flow cytometry at t= 144 hrs. Histogram plots are shown for 1 donor representative of 3.

Supp. Figure 5. HLA-A2 WT1-TCB-induced T cell activation upon first stimulation (24 hrs) in the assay described in figure 3 (E:T = 5:1). (**A, B**) The expression of CD25 and CD69 was measured on CD4+ and CD8+ T cells by flow cytometry. Mean of n = 3 donors +/- SD.

Supp. Figure 6. Effect of a non-targeting TCB (DP47-TCB) vs. HLA-A2 WT1-TCB on killing of SKM-1 tumor cells. NLR-labelled SKM-1 tumor cells were co-cultured with PBMCs and HLA-A2 WT1-TCB or DP47-TCB, E:T=2.5:1. Killing was followed by Incucyte (1 scan every 3 hrs, zoom 10x, phase and red 400 ms acquisition time). The percentage of inhibition of tumor cell killing was calculated over that in the absence of TCB for a fixed HLA-A2 WT1-TCB or DP47-TCB concentration of 10 μg/mL. Mean of n= 3 donors + SEM.

Supp. figure 7. Dasatinib prevents HLA-A2 WT1-TCB induced T cell degranulation. (**A**) PBMCs were co-cultured with SKM-1 target cells (E:T=5:1) and HLA-A2 WT1-TCB in the presence or absence of 100 nM dasatinib. Golgistop, Golgiplug and anti-CD107a antibody were added after 3 hrs of activation with the TCB. (**B**) Representative FACS plots of CD107a+ populations among CD4+ and CD8+ T cells for 1 donor representative of 3, 10 nM HLA-A2 WT1-TCB. (**C**)Percentages of CD107a+ cells among CD4+ and CD8+ T cells treated with 10 nM HLA-A2 WT1-TCB in the presence and absence of 100 nM

dasatinib. Mean of n = 3 donors +/- SD with with * $p \leq 0.05$, ** $p \leq 0.01$ by 1 way ANOVA (Friedman test).

Supp. Figure 8. Dasatinib prevents the release of IFN-γ and TNF-α by CD4+ and CD8+ T cells after stimulation with HLA-A2 WT1-TCB. PBMCs were co-cultured with SKM-1 target cells (E:T=5:1) and HLA-A2 WT1-TCB in the presence or absence of 100 nM dasatinib. Golgistop and Golgiplug were added 3 hours after activation with the TCB. (**A**, **C**) Representative FACS plots of IFN-γ and TNF-α populations among CD4+ and CD8+ T cells for 10 nM HLA-A2 WT1-TCB. (**B**, **D**) Percentages of IFN-γ- and TNF-α-positive cells among CD4+ and CD8+ T cells for 10 nM HLA-A2 WT1-TCB in the presence and absence of 100 nM dasatinib. Mean of n = 3 donors +/- SD with * $p \leq 0.05$, ** $p \leq 0.01$ by 1 way ANOVA (Friedman test).

Supp. Figure 9. (**A**) Effect of escalating concentrations of dasatinib on CEA-TCB-mediated cytokine release upon the first stimulation, in the assay described in figure 4 The levels of IFN-γ, TNF-α, IL-2, GM-CSF, IL-6 and IL-8 were measured in the supernatants of the assay at t=72 hrs by Luminex (1 donor representative of 2).

Supp. Figure 10. Low concentrations of dasatinib equilibrate TCB-induced cytokine release**.** (**A, B**) PBMCs were co-cultured with NLR-labelled MKN45 (E:T=10:1) target cells and 1 nM CEA-TCB for 2 consecutive stimulations in the presence or absence of 6.25 nM or 12.5 nM dasatinib upon the first stimulation. Real time killing was followed by Incucyte (1 scan every 3 hrs, zoom 10x, phase and red 400 ms acquisition time). Means of technical replicates +/-SD. (C) The levels of IFN-γ TNF-α and IL-2 were measured in the culture supernatant by Luminex after both stimulations (1 donor).

Supp. Figure 11. Effect of 100 nM dasatinib on CD19-TCB dependent T cell activation and cytokine release induced by CD19-TCB. PBMCs were co-cultured with CTV-labelled SU-DHL-8 tumor cells (E:T=10:1) and escalating concentrations of CD19-TCB in the presence or absence of 100 nM dasatinib. (A) The expression of CD25 and CD69 on CD4+ and CD8+ T cells was measured by flow cytometry and

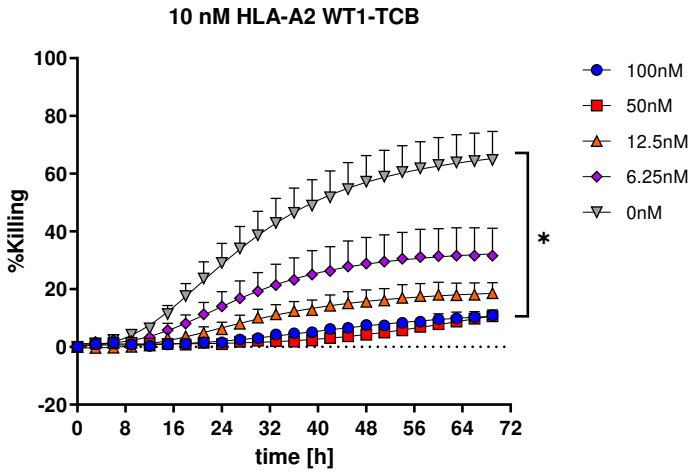
the levels of IFN-γ, TNF-α and IL-2 were measured by Luminex in the supernatants at t=24 hrs. Means of n = 3 donors +/- SD with *p ≤ 0.05, ** p ≤ 0.01 by 1 way ANOVA (Friedman test).

Supp. Documentation 1. Analysis reports for SKM-1 and MKN45 cell line typing from Microsynth.

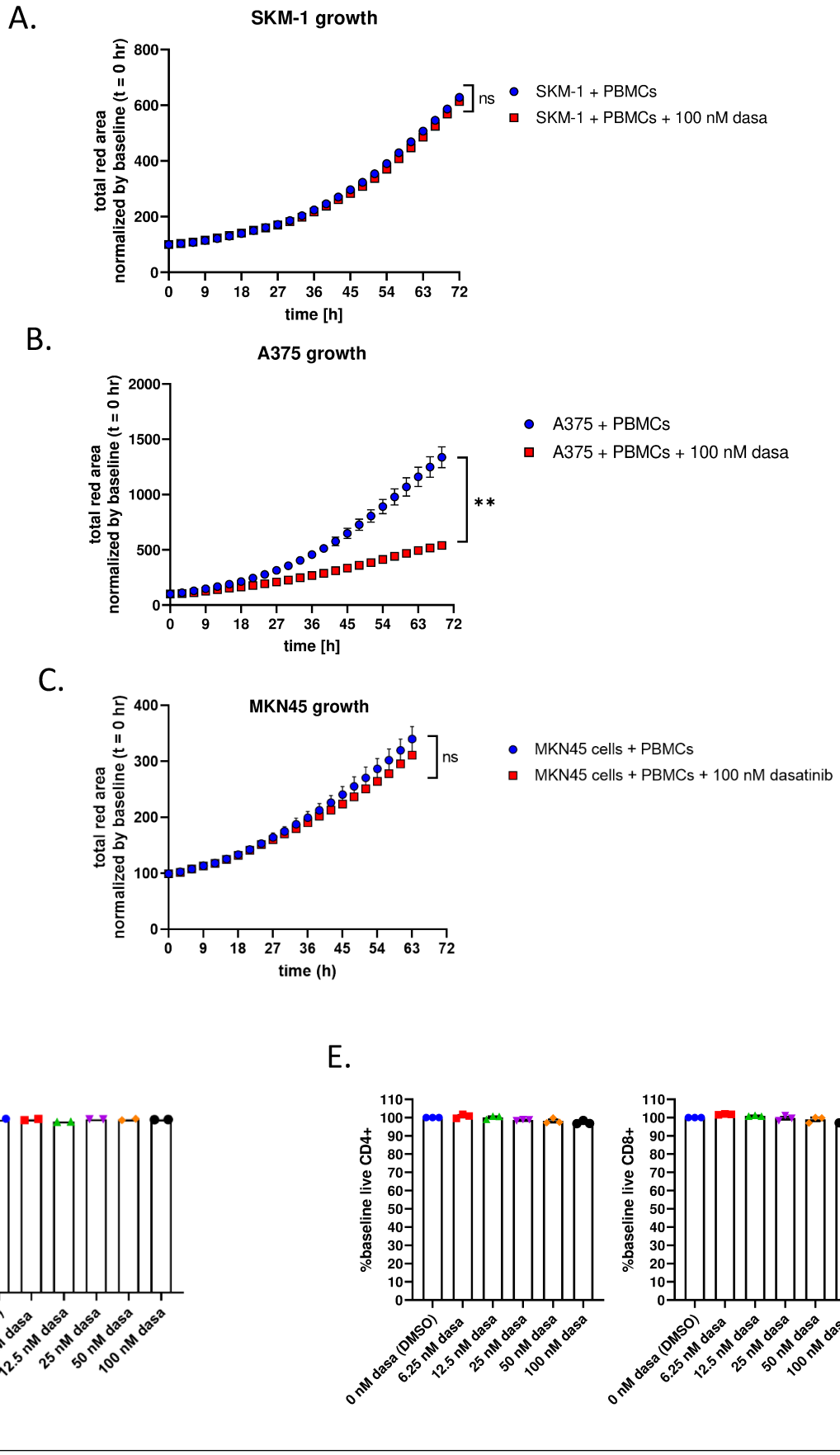
Supp. Video 1. The reversible effect of dasatinib on CEA-TCB-mediated killing of NLR-labelled MKN45 cells. Real time killing of NLR-labelled MKN45 cells by 1 nM CEA-TCB upon first stimulation in the absence of dasatinib (ON), second stimulation in the presence of dasatinib (OFF) and third stimulation in the presence of dasatinib (OFF), as shown on Fig 4. Incucyte video (1 scan every 3 hrs, zoom 10x, phase and red, 400 ms acquisition time) for 1 donor representative of 3.

Leclercq G, *et al. J Immunother Cancer* 2021; 9:e002582. doi: 10.1136/jitc-2021-002582

Supp. Figure 1



Supp. Figure 2



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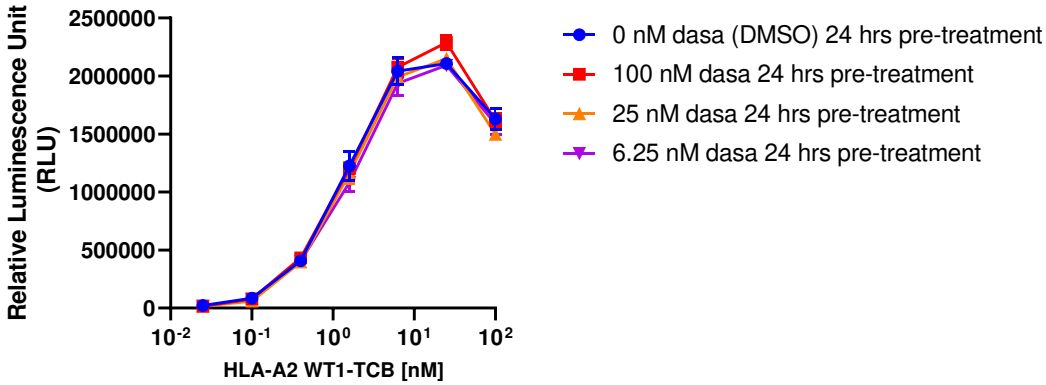
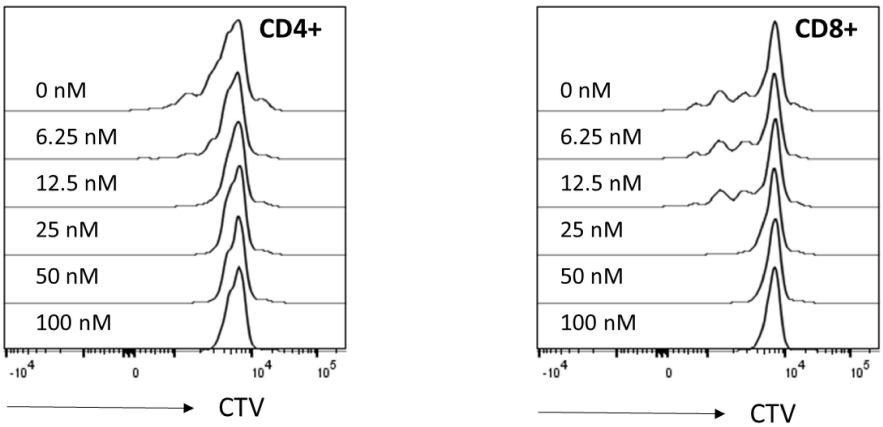


Supp. Figure 4

A.

Donor 2



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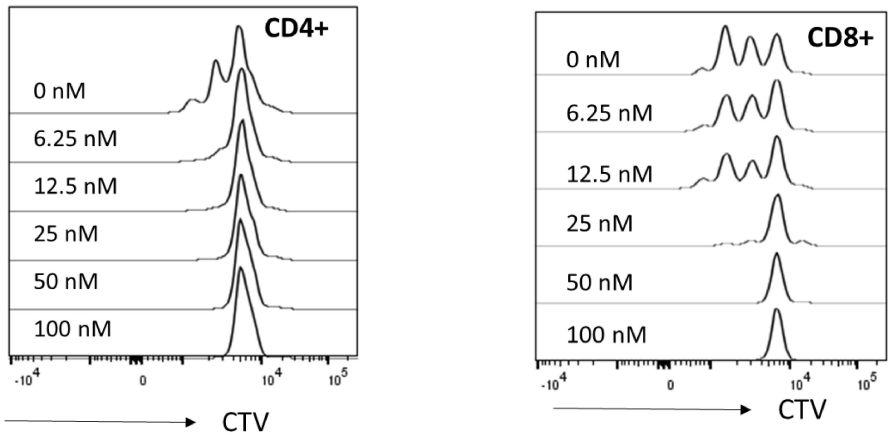

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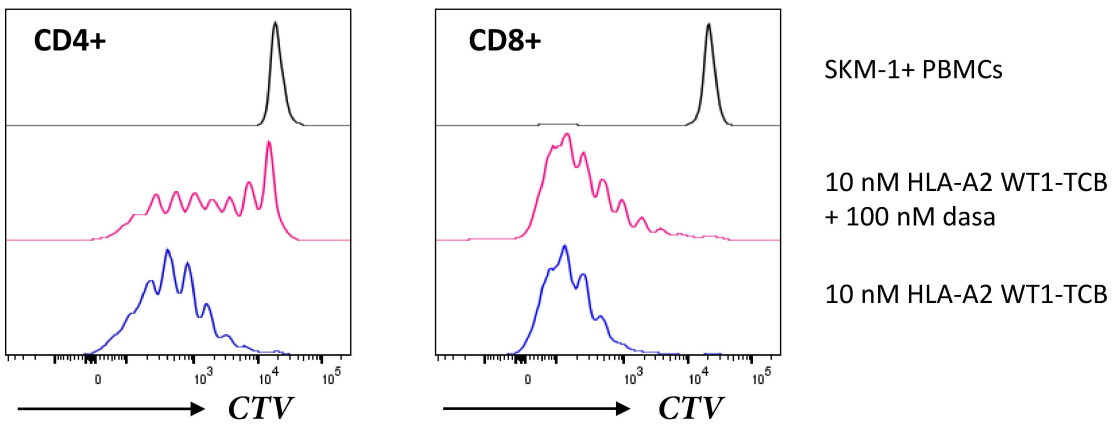

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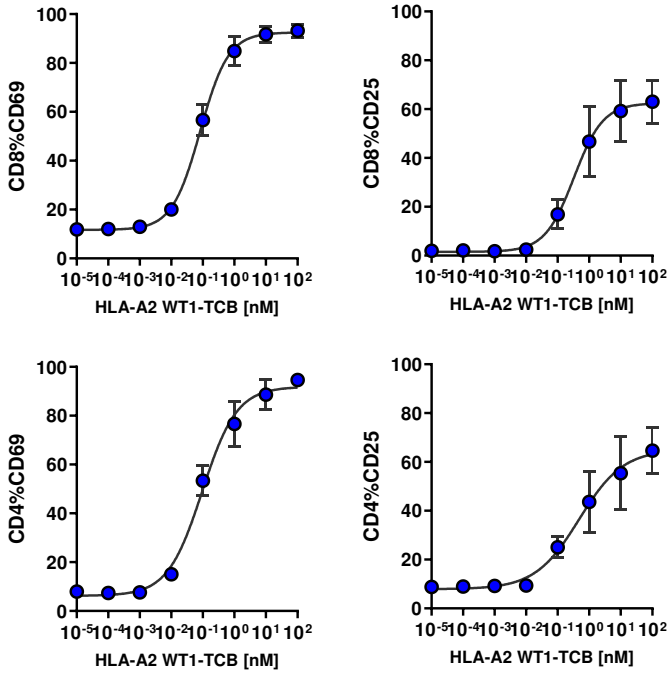

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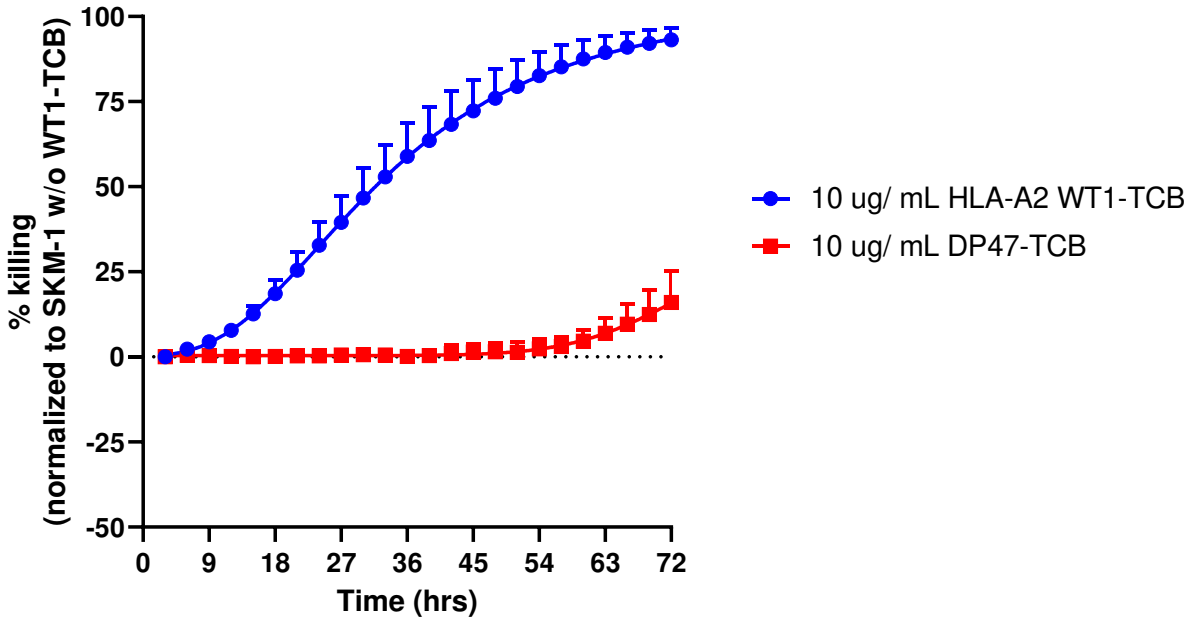

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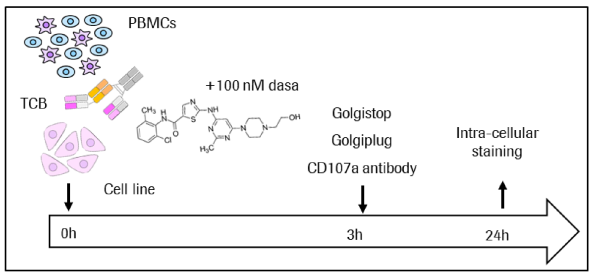
A.



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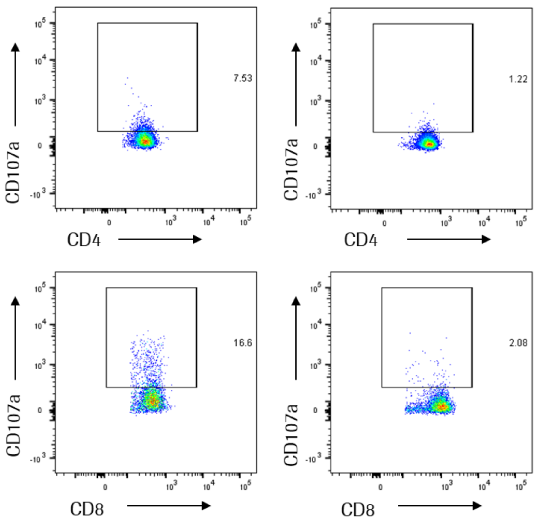

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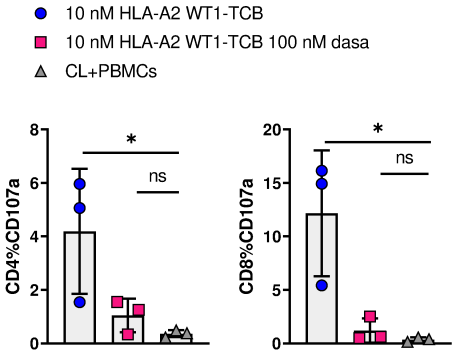

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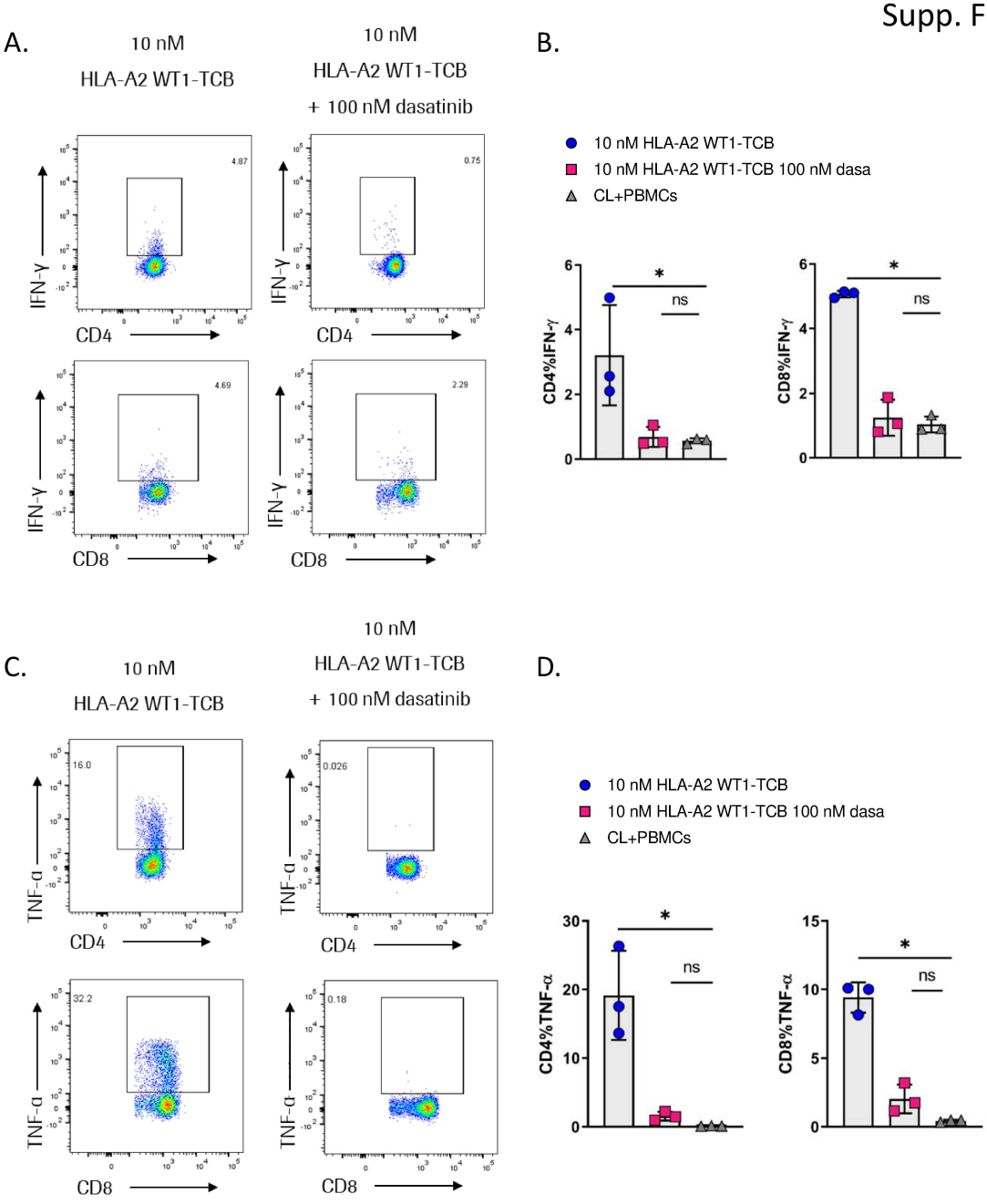

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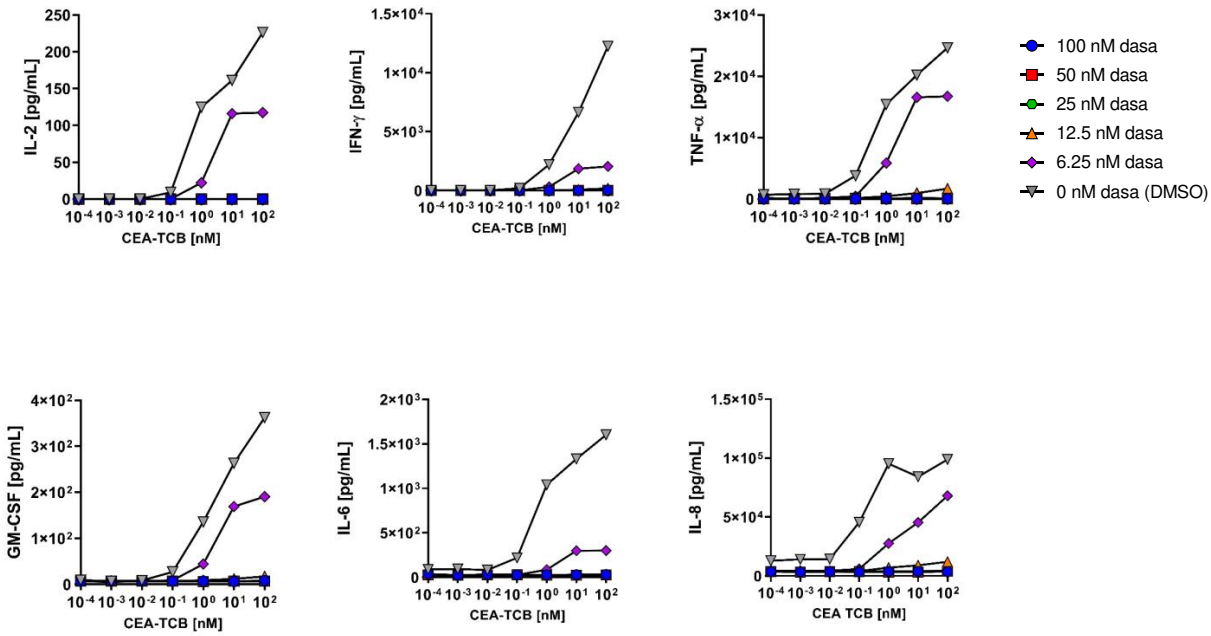
A.



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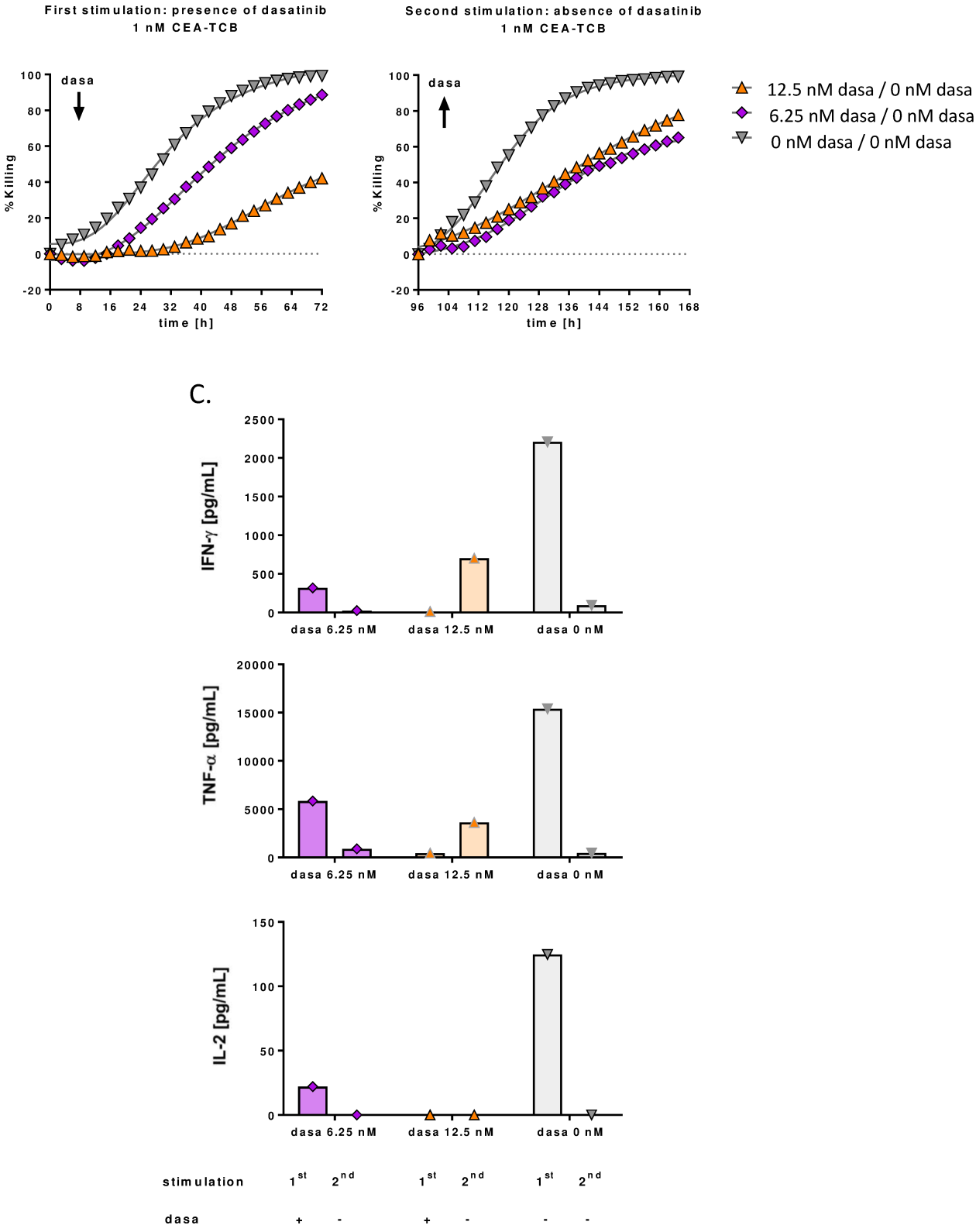

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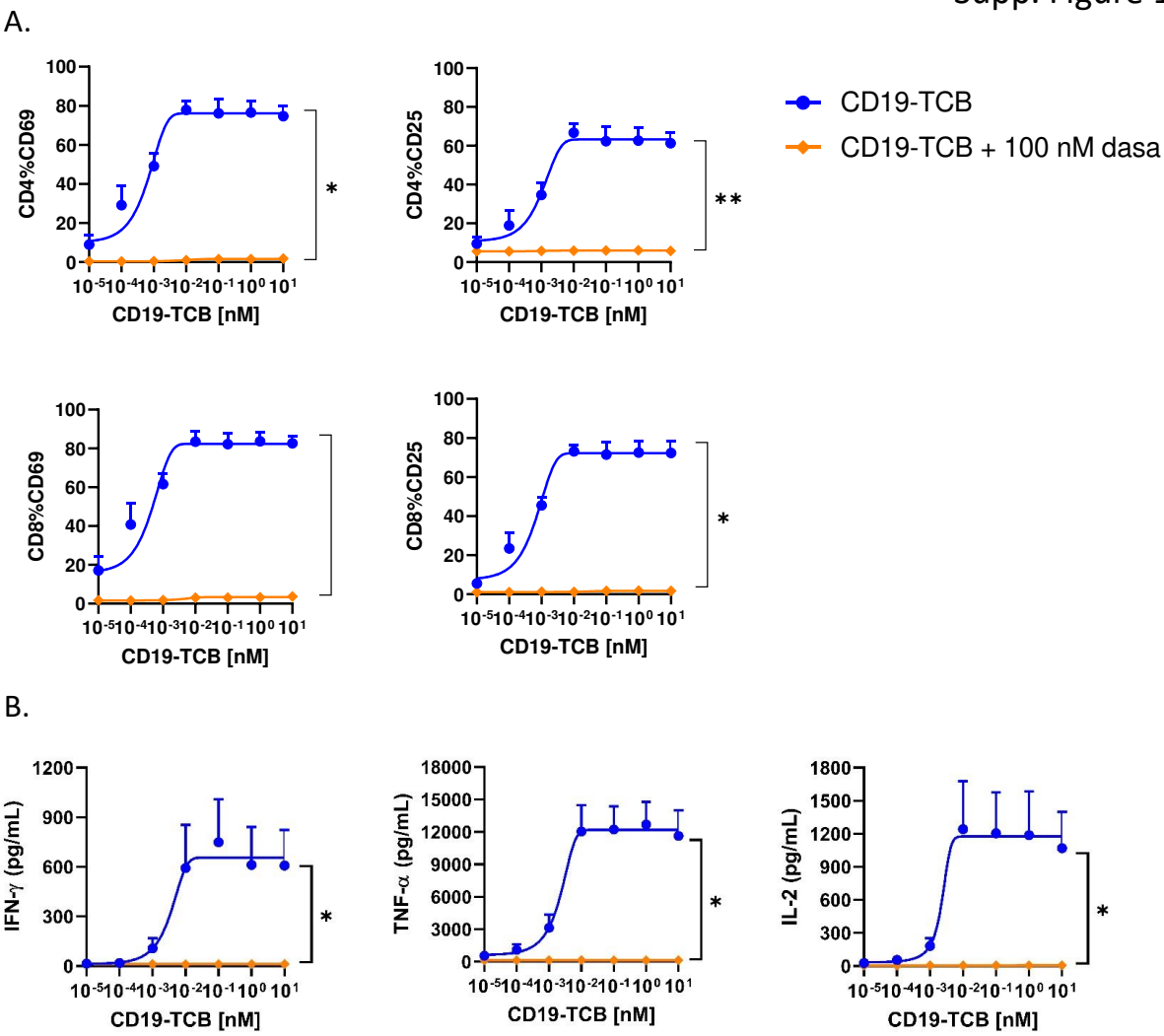


Microsynth

ANALYSIS REPORT
Human Cell Line Typing

Analysis Report for Cell Line Typing

1. Sponsor

Mr. Christian Pohl
Roche Glycart AG
Wagistrasse 10
8952 Schlieren

2. Analysis Report

Report ID: 01224_007044
Report Version: 01
Issue Date: 23.04.2021
Report Approved: Joy Beyer

3. Descriptions

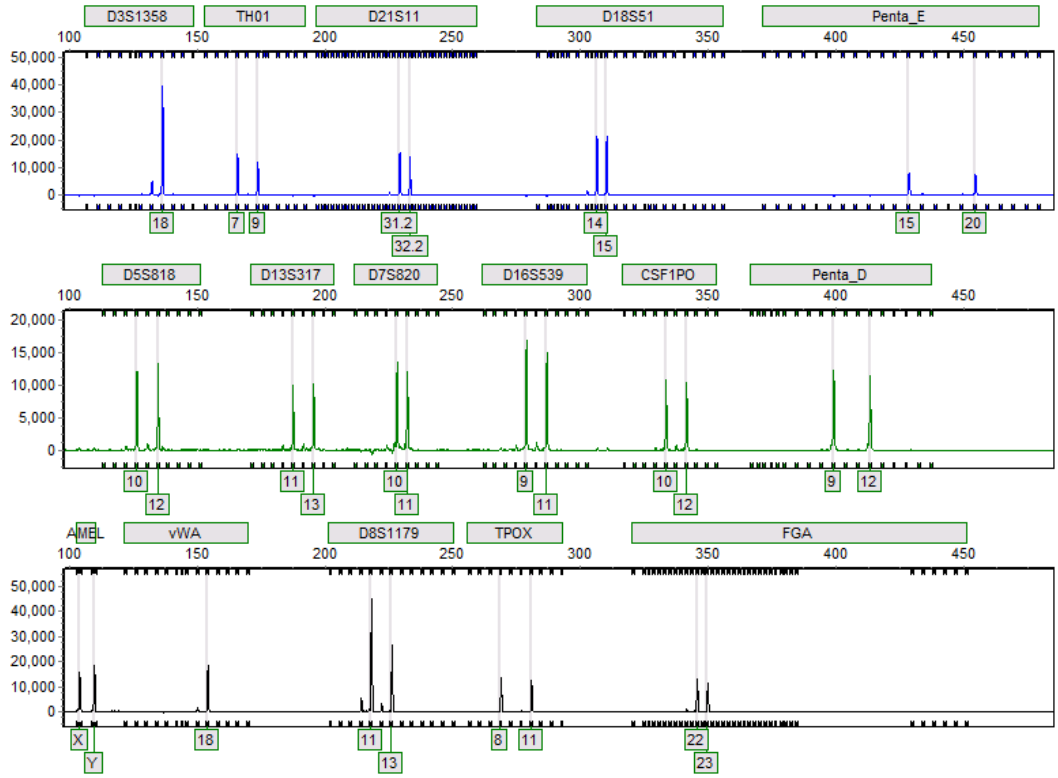
Customer Test Item ID: 20210419_SKM1
Analysis Method: Profiling of the human cell lines was done using highly polymorphic short tandem repeat loci (STRs). STR loci were amplified using the PowerPlex® 16 HS System (Promega). Fragment analysis was done on an ABI3730xl (Life Technologies) and the resulting data were analyzed with GeneMarker HID software (Softgenetics).

4. Analysis Results

4.1. Summary Table of the STR Profile

Locus	*Chromosomal Location*	*Core STR Marker*	*Customer Sample Typed Alleles*	*Database Alleles*	*Comments*
D3S1358	Chr03		18	18	
TH01	Chr11	Yes	7/9	7/9	
D21S11	Chr21		31.2/32.2	31.2/32.2	
D18S51	Chr18		14/15	14/15	
Penta_E	Chr15		15/20	15/20	
D5S818	Chr05	Yes	10/12	10/12	
D13S317	Chr13	Yes	11/13	11/13	
D7S820	Chr07	Yes	10/11	10/11	
D16S539	Chr16	Yes	9/11	9/11	
CSF1PO	Chr05	Yes	10/12	10/12	
Penta_D	Chr21		9/12	9/12	
AMEL	X/Y	Yes	X/Y	X/Y	
vWA	Chr12	Yes	18	18	
D8S1179	Chr08		11/13	11/13	
TPOX	Chr2	Yes	8/11	8/11	
FGA	Chr04		22/23	22/23	

4.2. Electropherogram



5. Conclusion

According to our analysis of the submitted sample there is no detectable contamination with human origin.

The analyzed data of the submitted sample match 100 % to the DNA profile of the cell line SKM-1 (Cellosaurus, RRID:CVCL_0098).

6. Customer Comment

No specific customer comments were provided for this test item

7. Glossary

Short Tandem Repeats (STRs)

Short tandem repeats (STRs) consist of a DNA motif of 2-13 bases that are repeated up to several hundred times. The number of repeats in a STR is highly variable among individuals, resulting in fragment length differences if amplified using PCR. These differences in fragment lengths at different loci are used for profiling the cell lines.

Stutter Peaks

Stutter peaks are small peaks which occur immediately before or after the true peak. Stutter peaks are commonly caused by a slippage of the polymerase during the PCR amplification.

Detection of Cell Line Mixtures

Contamination of one cell line by one or several other cell lines can be detected down to a frequency of the contaminating cell line of 10%. Typically, cell line mixtures will result in STR profiles including three or more peaks for single or multiple loci. If Microsynth notices a possible contamination of a cell line, we will comment the finding in the conclusion part of the analysis.

Peak height ratio

Peak height ratio <25 % (to the highest peak within a STR) is mentioned in the summary table (comments). Peak height ratios <25% need not necessarily have an effect on the behaviour or characteristics of the cell line. A small peak height may be due to reduced amplification efficiency, for example resulting from a mutation in the primer site. The reason for the difference in peak heights observed, however, would need some in depth analysis of the test item.

8. General Comment

The results refer only to the portion of the sample Microsynth has analyzed. The analysis results might not be assigned unconditionally to the whole sample. Microsynth shall not in any event be liable for incidental, consequential or special damages in relation to carried out analyses and corresponding results.

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9. Compliance and Quality Assurance Statement

All aspects of this study were in accordance with ISO 9001:2015 standards. All the applied equipment is qualified and calibrated. The applied methods are validated.

Leclercq G, *et al. J Immunother Cancer* 2021; 9:e002582. doi: 10.1136/jitc-2021-002582

Microsynth

ANALYSIS REPORT
Human Cell Line Typing

Analysis Report for Cell Line Typing

1. Sponsor

Mr. Christian Pohl
Roche Glycart AG
Wagistrasse 10
8952 Schlieren

2. Analysis Report

Report ID: 01224_007045
Report Version: 01
Issue Date: 23.04.2021
Report Approved: Joy Beyer

3. Descriptions

Customer Test Item ID: 20210419_MKN45
Analysis Method: Profiling of the human cell lines was done using highly polymorphic short tandem repeat loci (STRs). STR loci were amplified using the PowerPlex® 16 HS System (Promega). Fragment analysis was done on an ABI3730xl (Life Technologies) and the resulting data were analyzed with GeneMarker HID software (Softgenetics).

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4.1. Summary Table of the STR Profile

Locus	*Chromosomal Location*	*Core STR Marker*	*Customer Sample Typed Alleles*	*Database Alleles*	*Comments*
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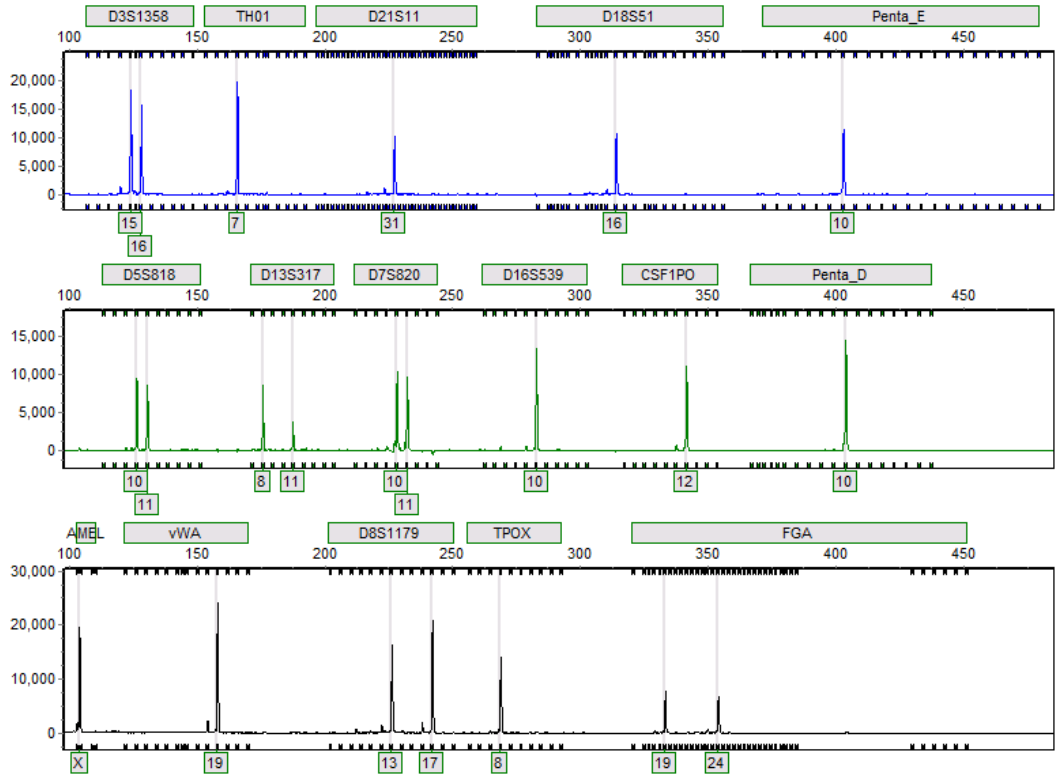
D13S317	Chr13	Yes	8/11	8/11	
D7S820	Chr07	Yes	10/11	10/11	
D16S539	Chr16	Yes	10	10	
CSF1PO	Chr05	Yes	12	12	
Penta_D	Chr21		10	10	
AMEL	X/Y	Yes	X	X	
vWA	Chr12	Yes	19	19	
D8S1179	Chr08		13/17	13/17	
TPOX	Chr2	Yes	8	8	
FGA	Chr04		19/24	19/24	

4.2. Electropherogram



5. Conclusion

According to our analysis of the submitted sample there is no detectable contamination with human origin.

The analyzed data of the submitted sample match 100 % to the DNA profile of the cell line MKN45 (Cellosaurus, RRID:CVCL_0434).

6. Customer Comment

No specific customer comments were provided for this test item

7. Glossary

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