$BRAF^{V600E}$ expression in hematopoietic progenitors leads to myeloid skewing and histiocytosis

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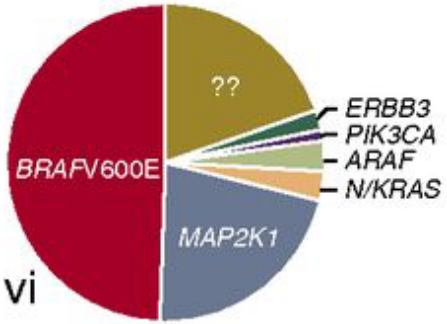
$BRAF^{V600E}$ is frequently detected in patients with L group histiocytoses

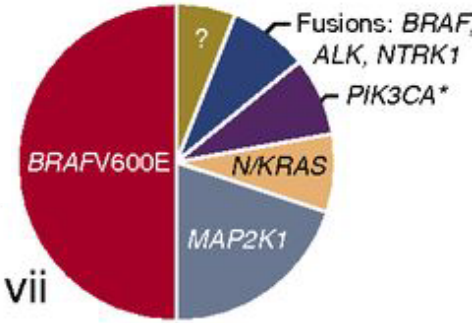
L Group

- LCH
- ICH
- ECD
- Mixed LCH/ECD

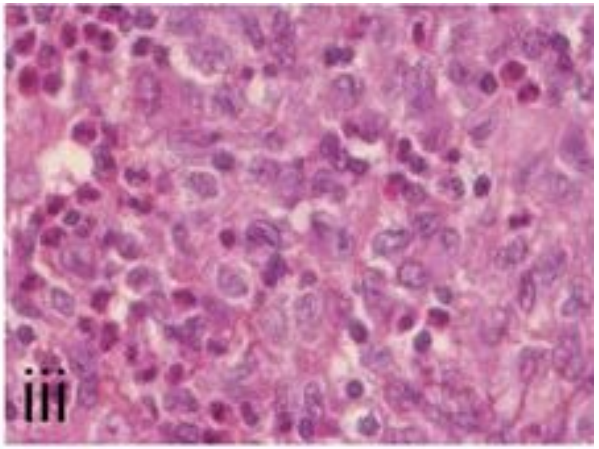
Langerhans Cell Histiocytosis

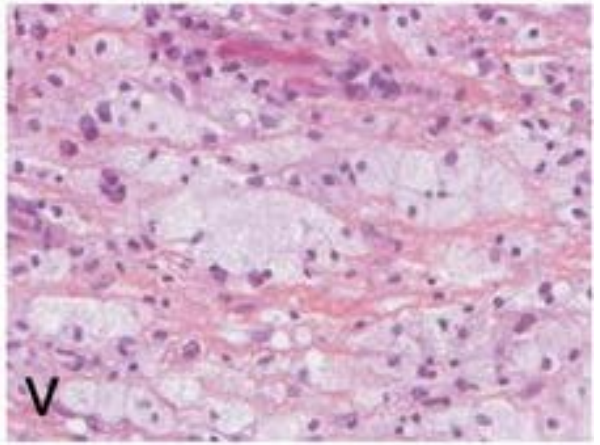


Erdheim-Chester Disease



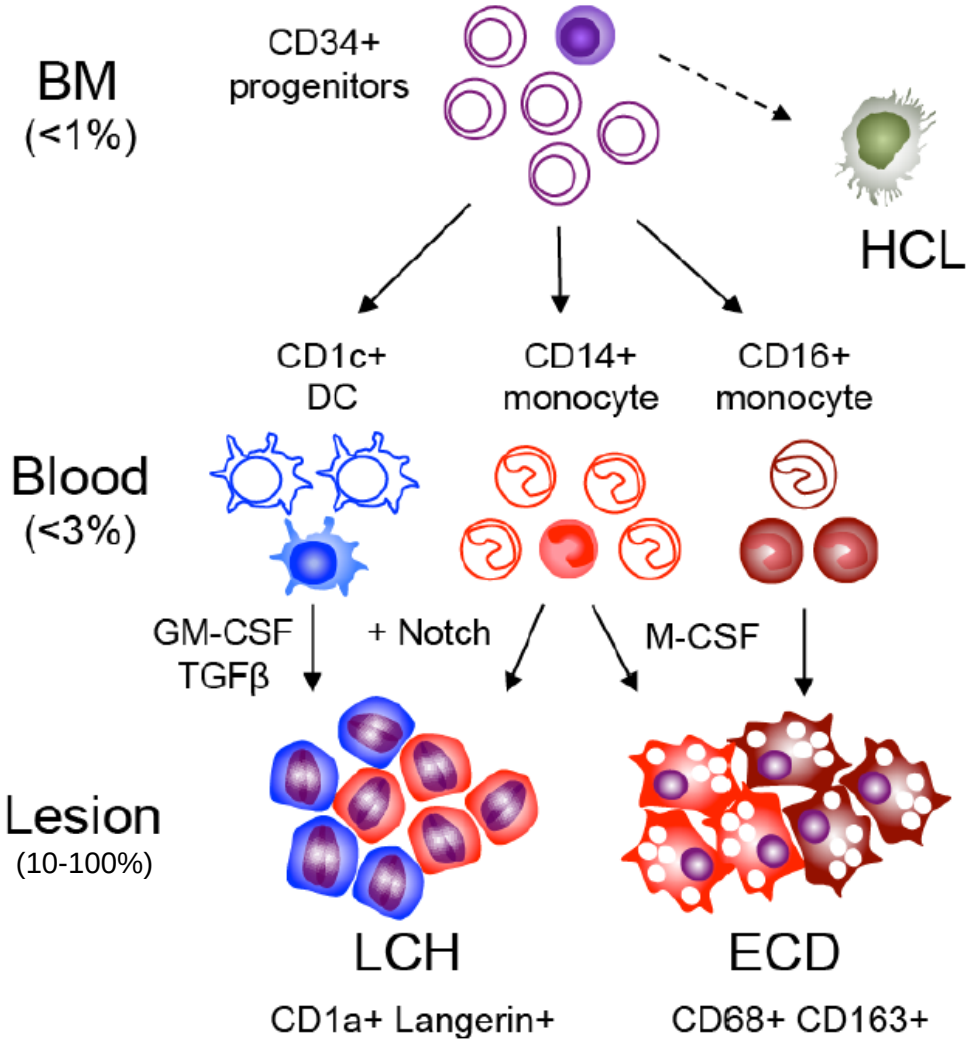
* A proportion of *PIK3CA* mutant patients have concomitant *BRAF*V600E mutations.





Emile *et al* Blood 2016

$BRAF^{V600E}$ can be found in histiocytes, monocytes and HSPCs from histiocytosis patients



Milne *et al* Blood 2017; Berres *et al* J Exp Med 2014

Aim of the project

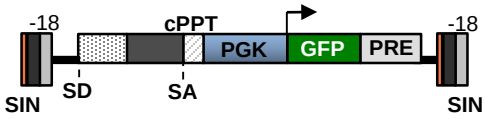
To model and characterize the impact of $BRAF^{V600E}$ expression on histiocytosis development we would need:

- Human HSPCs as cells-of-origin
- Low percentage of $BRAF^{V600E}$–mutated cells to recreate human situation
- Experimental conditions that allow histiocyte differentiation and maturation

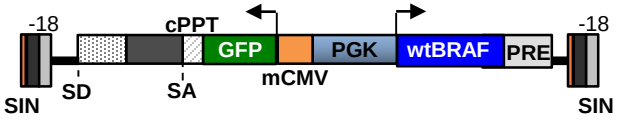
Experimental strategy

Lentiviral vector constructs

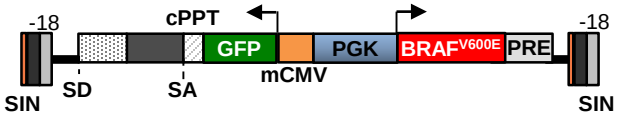
GFP



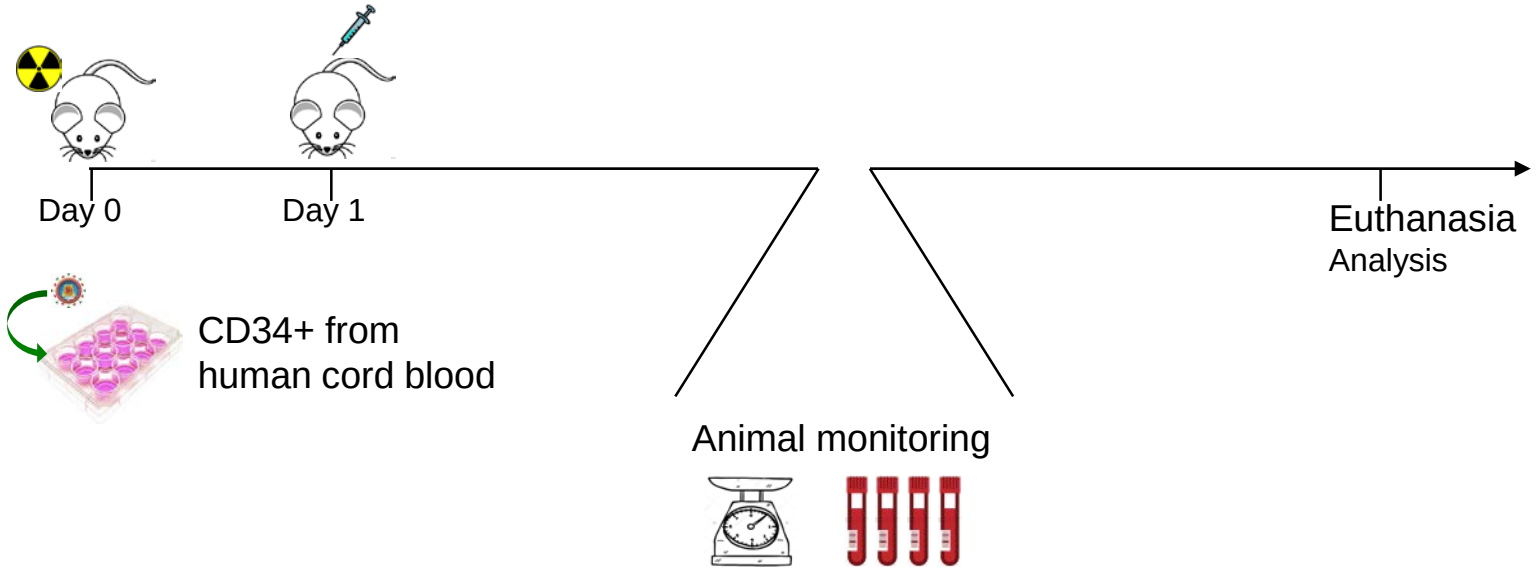
wtBRAF



$BRAF^{V600E}$

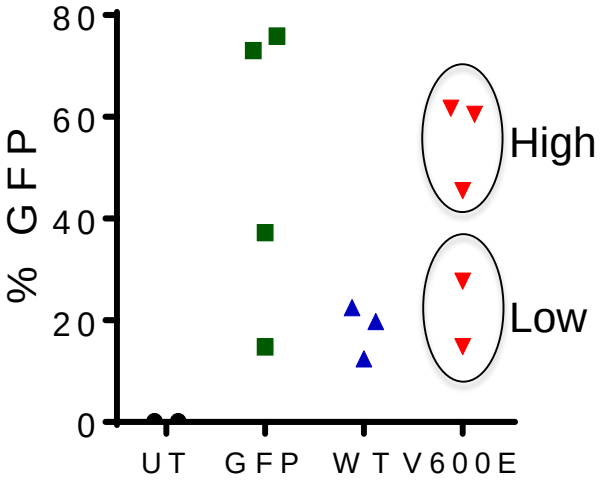


NOD/SCID-γchain^{null} (NSG) mouse

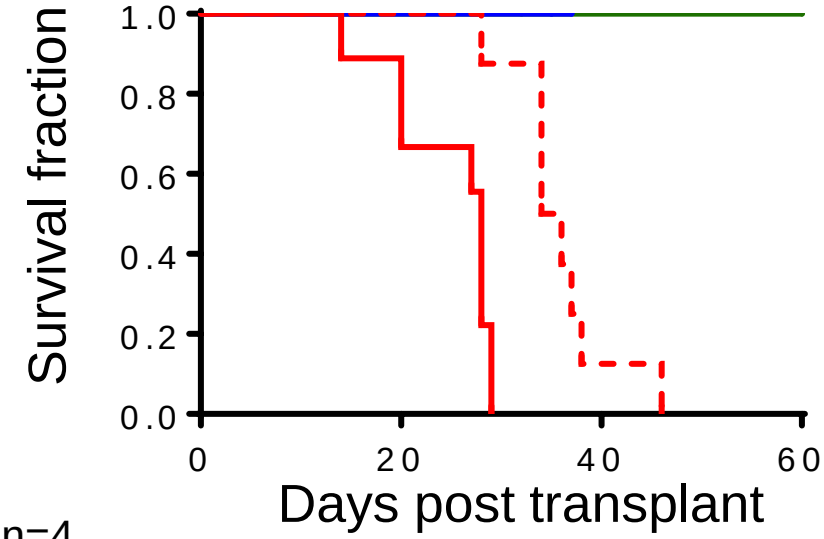


Mice show $BRAF^{V600E}$–dose dependent survival

In vitro transduction



Survival curve



UT n=4
GFP n=13
WT n=8
V600E low n=8
V600E high n=8

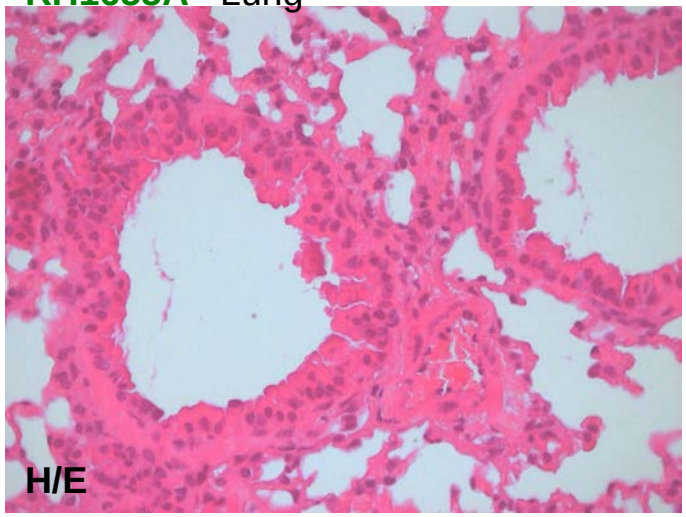
Pathology analyses show multisystemic infiltration of large mononuclear cells

Infiltration grade

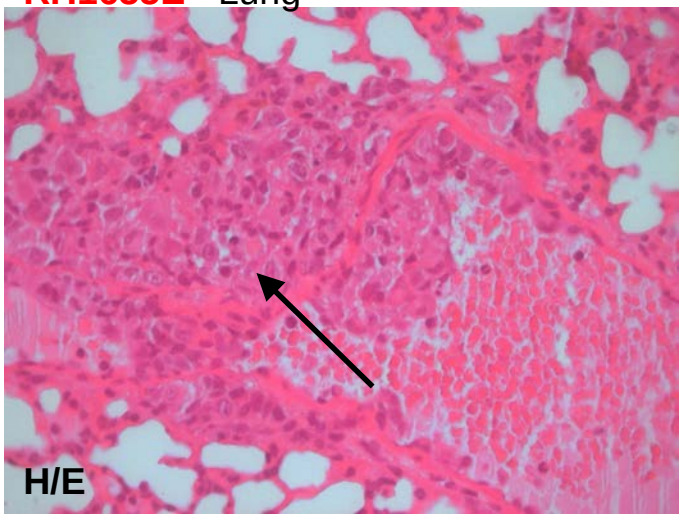
0 3+

Code	Vector	Spleen	Liver	Lung	Kidney	Heart	Thymus	Brain	Meninges	Gut	BM femur	BM sternum
RH1655A	GFP	0	0	0	0	0	0	0	0	0	0	0
RH1655B		0	0	0	0	0	0	0	0	0	0	0
RH1655C		0	0	0	0	0	0	0	0	0	0	0
RH1656C	UT	0	0	0	0	0	0	0	0	0	0	0
RH1656D		0	0	0	0	0	0	0	0	0	0	0
RH1656E	wtBRAF	0	0	0	0	0	0	0	0	0	0	0
RH1656F		0	0	0	0	0	0	0	0	0	0	0
RH1656A		0	0	0	0	0	0	0	0	0	0	0
RH1656B		0	0	0	0	0	0	0	0	0	0	0
RH1655D	$BRAF^{V600E}$	1	0	1	1	0	NA	0	1+	0	3	3+
RH1655E		NA	1	2	1+	0	NA	0	1+	0	3	3+
RH1655F		0	0	0	0	0	0	0	0	0	2+/3+	2+/3+

RH1655A - Lung



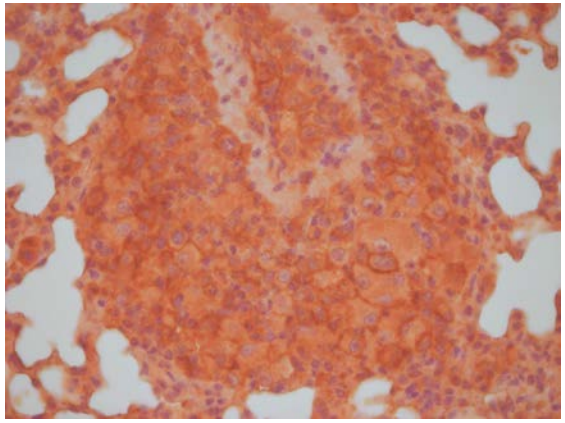
RH1655E - Lung



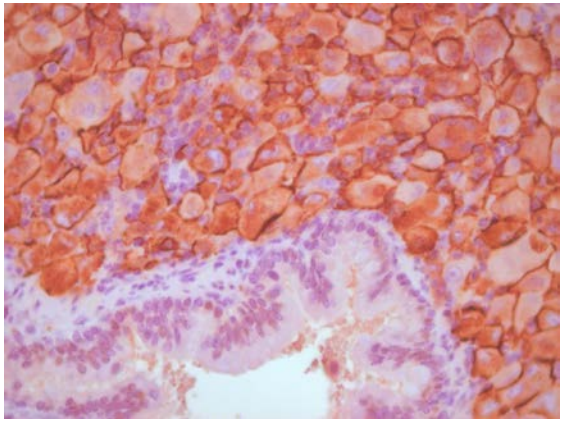
In collaboration with Maurilio Ponzoni

Infiltrating cells display histiocytosis immunophenotype

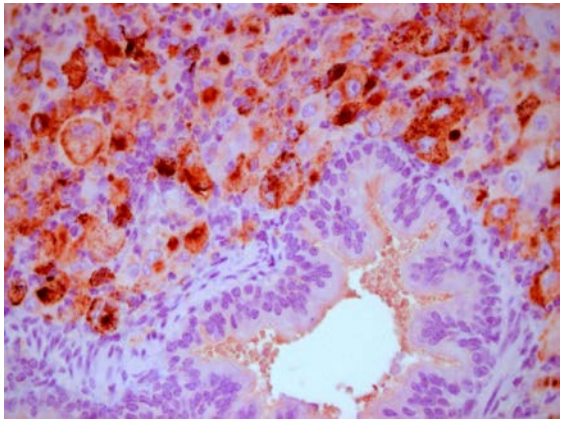
CD33



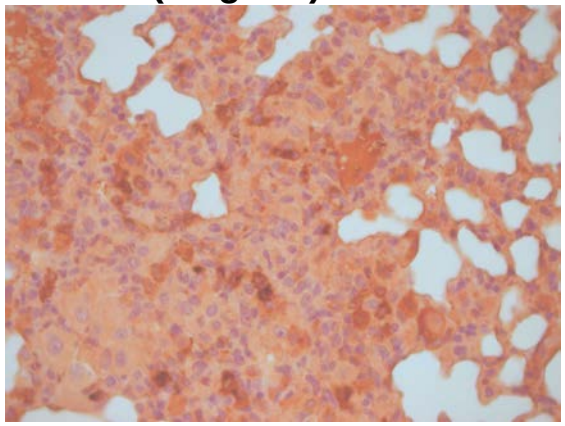
CD14



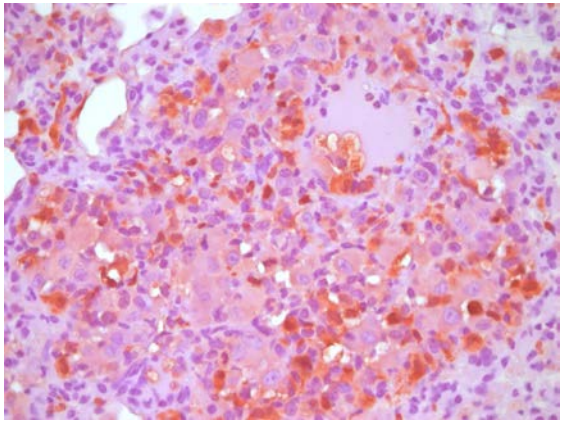
CD68



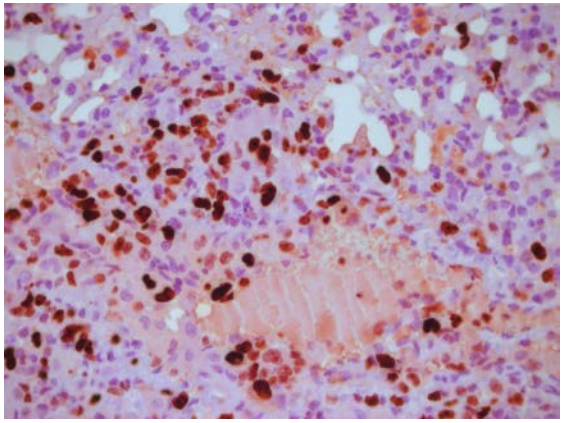
CD207 (langerin)



S100

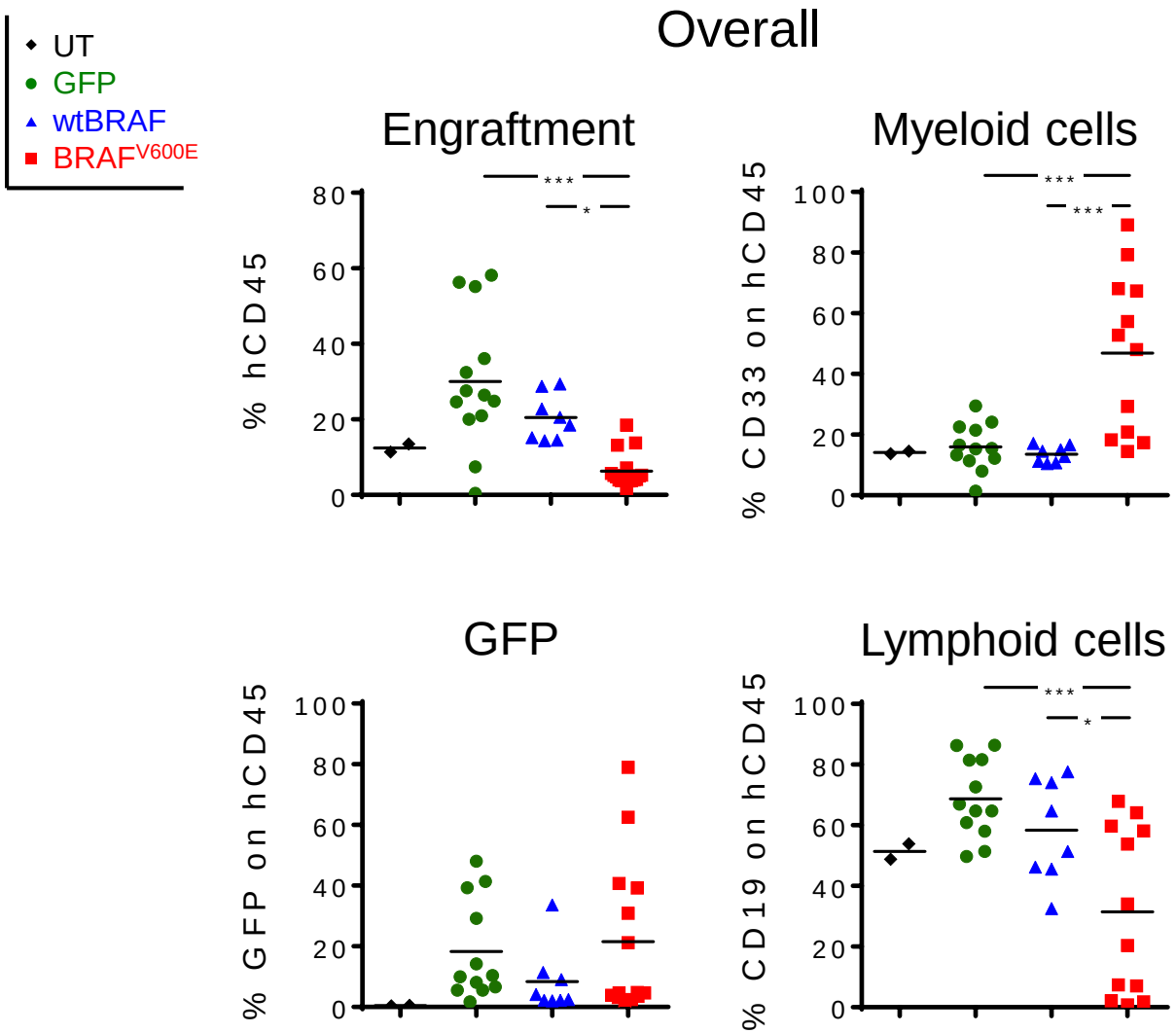


Ki67



In collaboration with Maurilio Ponzoni

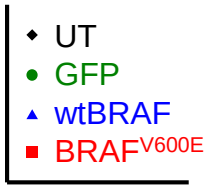
$BRAF^{V600E}$ impairs engraftment and induces myeloid skewing



1-way ANOVA

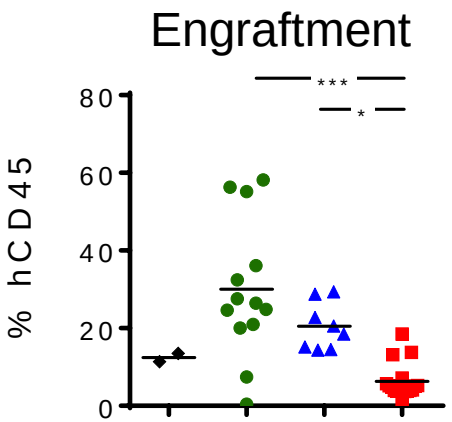
*** p<0,001

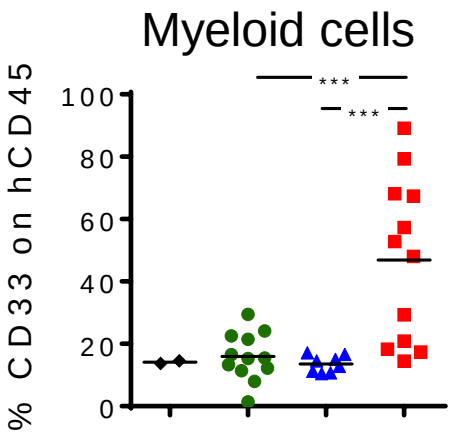
Myeloid skewing is BRAF^{V600E}-dose dependent

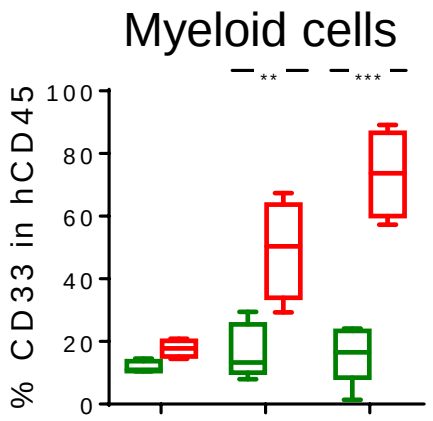


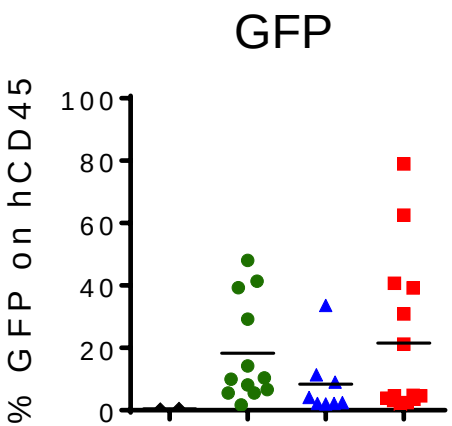
Overall

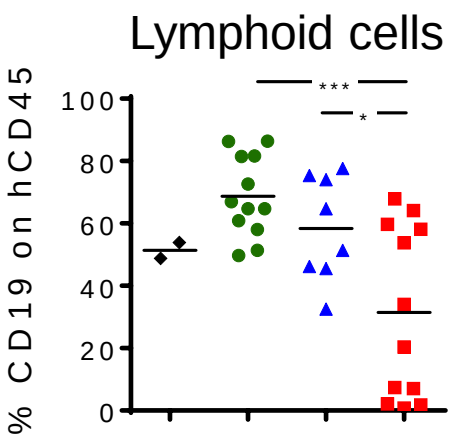
Stratification by *in vitro* transduction level

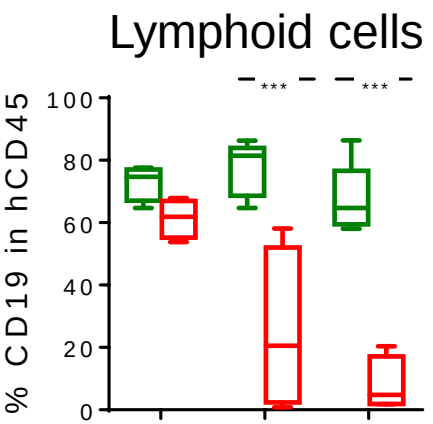








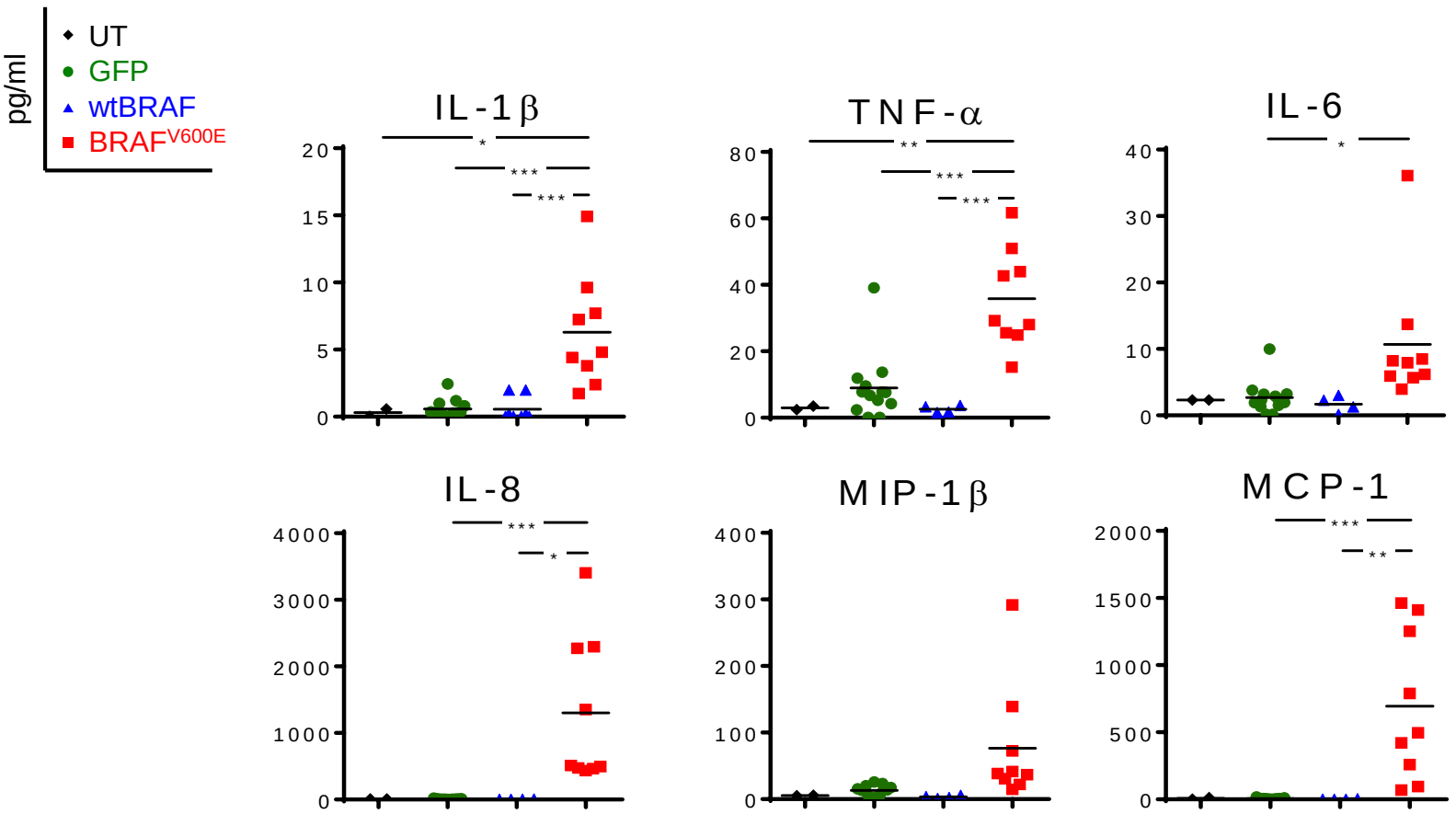




1-way ANOVA

*** p<0,001

Plasma level of proinflammatory cytokines recapitulate patient phenotype



1-way ANOVA

* p<0,05

** p<0,01

*** p<0,001

$BRAF^{V600E}$ promotes survival and differentiation in primary human monocytes *in vitro*

Materials:

10^6 healthy donor monocytes

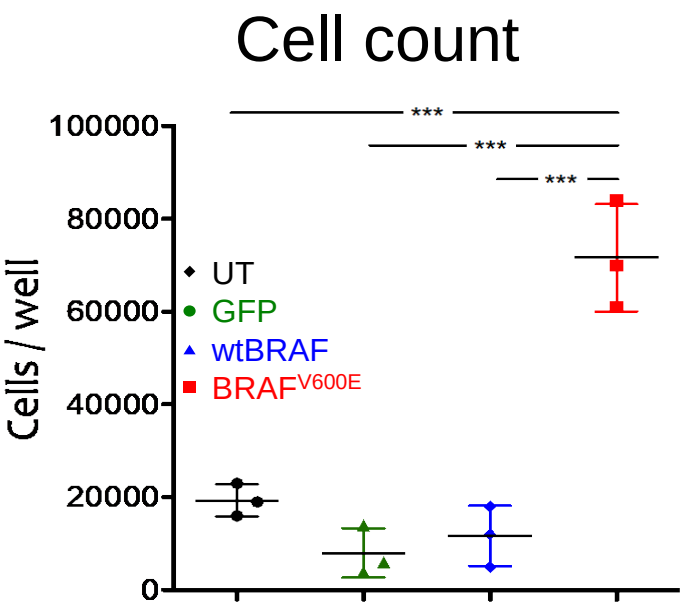
RPMI + 10% FBS + 5% human serum for 2 weeks

LV MOI 1: $BRAF^{V600E}$ titer $2{,}19*10^9$ TU/ml, infectivity $2{,}97*10^4$ TU/ng

wtBRAF titer $6{,}44*10^9$ TU/ml, infectivity $5{,}97*10^4$ TU/ng

GFP titer $4{,}5*10^9$ TU/ml, infectivity $7{,}42*10^4$ TU/ng

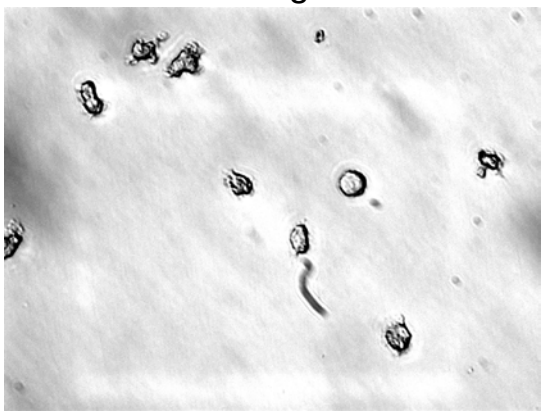
Vpx-VLP



1-way ANOVA

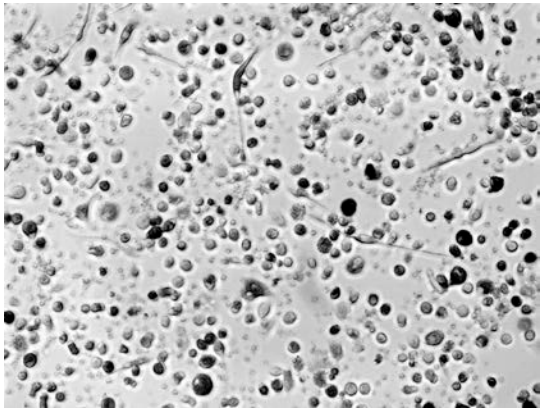
*** p<0,001

wtBRAF 40X brightfield

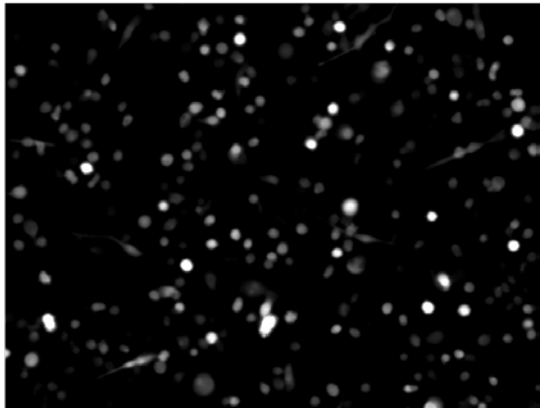


$BRAF^{V600E}$ 40X brightfield

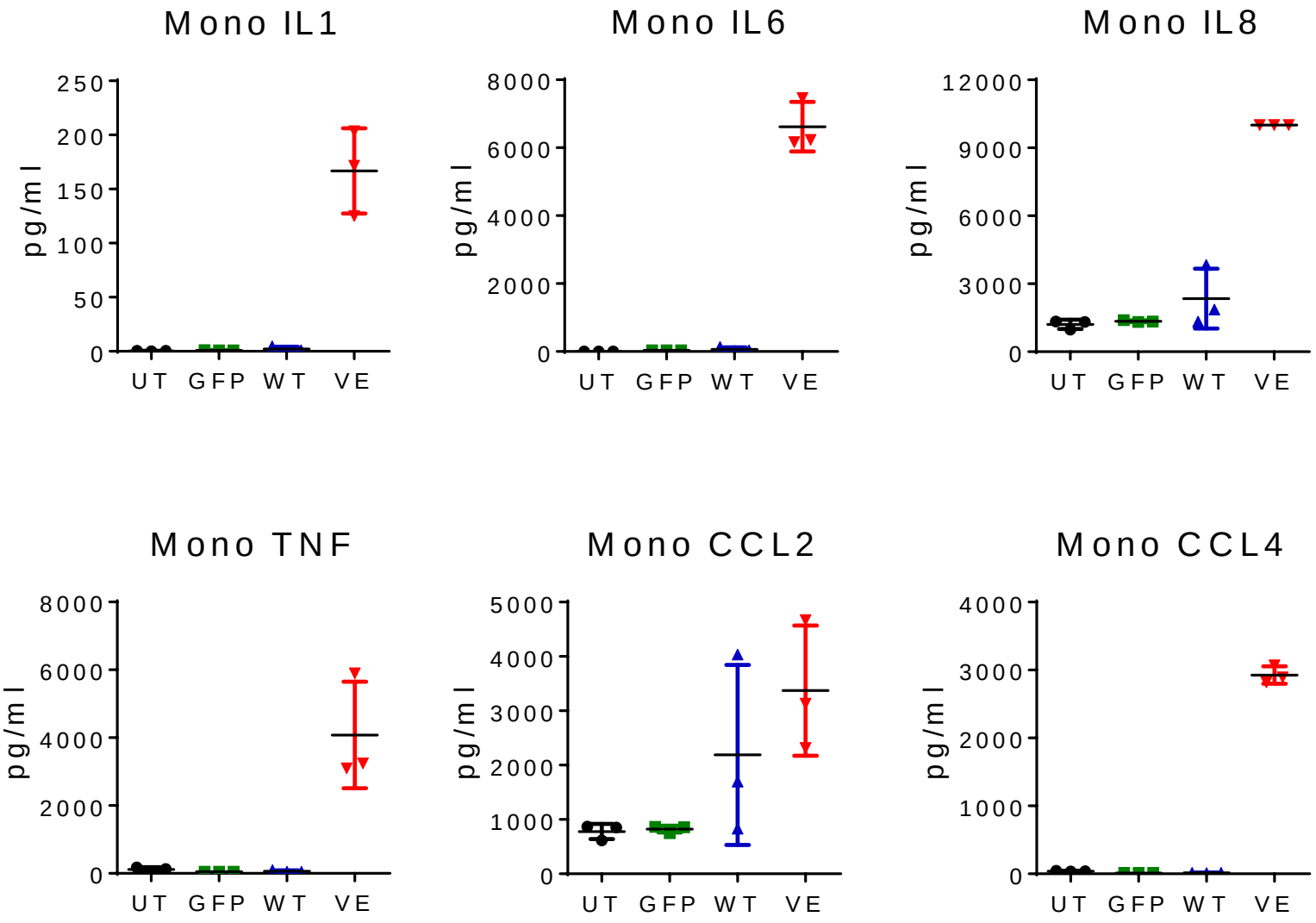
$BRAF^{V600E}$ 20X brightfield



$BRAF^{V600E}$ 20X GFP



$BRAF^{V600E}$ promotes spontaneous cytokine production in primary human monocytes *in vitro*



Conclusions and future perspectives

BRAF^{V600E} expression in human HSPCs transplanted in NSG mice induces a **lethal histiocytosis** phenotype

- **Histiocytes disseminate** in various organs and display LCH immunophenotype
- **Proinflammatory cytokines** are elevated in the plasma of mice
- BRAF^{V600E} expression **skews hematopoiesis** towards myeloid lineage

We will express BRAF^{V600E} in **different progenitor subpopulations** to identify ECD and LCH cell of origin

This model will help us characterize the **biologic effects of BRAF^{V600E}** on histiocytes, monocytes and hematopoietic progenitors

- **Drug test** to refine preclinically current therapetic strategies
- **RNA-seq** to identify new druggable targets
- Study the role of **maladaptive trained immunity** as potential disease mechanism

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