UFRJ

EuroFlow

STANDARIZED PANEL AND PROCEDURES FOR PEDIATRIC SOLID TUMORS DIAGNOSTIC BY FLOW CYTOMETRY

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UFRJ-Rio de Janeiro/Brazil

ESCCA
European Society
for Clinical Cell Analysis

ESCCA, Belfast, Sep.23rd.2022

Pediatric cancer is the 1st cause of death by diseases in children >1 year-old in developed countries


LIFE&STYLE | FUGAS | ÍPSILON | GUIA DO LAZER | CINECARTAZ | INIMIGO PÚBLICO | P3 |

Público

Vídeo
Passo-a-passo para preparar um rolo de sushi

Fotogaleria
Unidos contra o ACTA

JORNAL DO DIA | VÍDEOS | MULTIMÉDIA | INFOGRAFIAS | BLOGUES | LOJA | ASSINATUR

MUNDO POLÍTICA ECONOMIA DESPORTO SOCIEDADE EDUCAÇÃO CIÊNCIAS ECOSFERA CULTUR

Saúde

Cancro é principal causa de morte natural em crianças com mais de um ano

10.02.2010 - 16:11 Por Catarina Gomes

Votar ★★★★★ | 0 votos ★★★★★ +1 0 Gosto 9 de 16 notícias em Sociedade « anterior seguinte

O cancro pediátrico é uma doença rara mas "é a principal causa de morte natural em crianças com mais de um ano em Portugal", afirmou hoje a oncologista pediátrica do Instituto Português de Oncologia de Lisboa, Gabriela Caldas, que apresentou em Lisboa um estudo sobre o tema. Em primeiro lugar na mortalidade surgem os acidentes.

Childhood and Adolescent Cancer Statistics, 2014

Ward, PhD[1]; Carol DeSantis, MPH[2]; Anthony Robbins, MD, PhD[3]; Betsy Kohler, MPH[4]; Ahmedin Jemal, DVM,

In 2014, the American Cancer Society reported:

- **15780 new cases of pediatric cancer in USA**
- **1960 deaths**
- **Annual incidence of 18.6 per 1 million (since birth to 19 years)**

Ward E et al. Cancer J Clin 2014; 64:83-103

Pediatric Cancer Distribution

CA CANCER J CLIN 2014;64:83–103

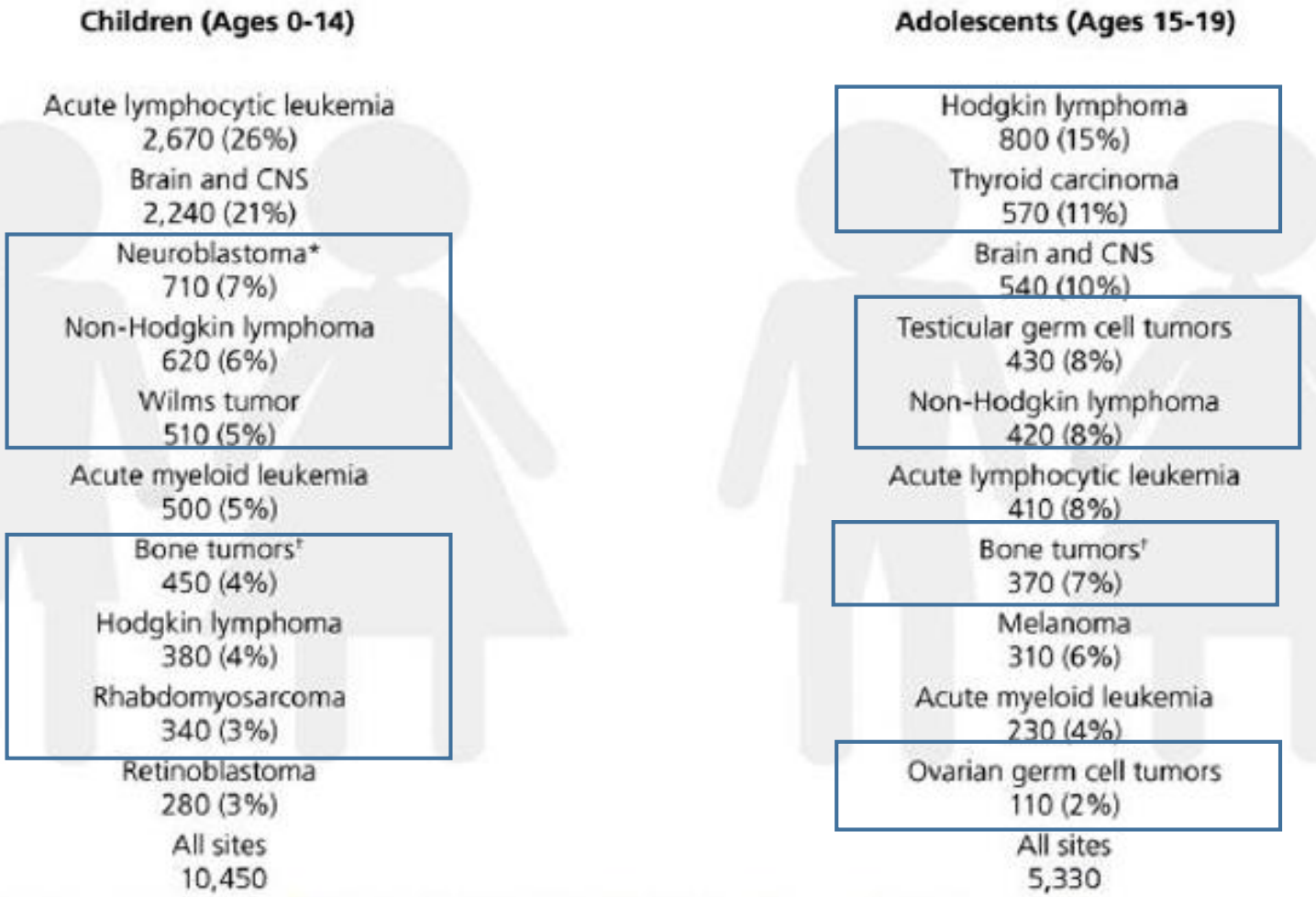

FIGURE 1. Estimated New Cases of Childhood and Adolescent Cancers, United States, 2014.
Estimates are for malignant cancers only and are rounded to the nearest 10. In addition, 730 children and 630 adolescents will be diagnosed with benign and borderline brain tumors in 2014. *Includes ganglioneuroblastoma. †Bone tumors include osteosarcoma and Ewing sarcoma.

Ward et al. Cancer J Clin, 2014

Clinical suspicion and conventional diagnosis in pediatric solid tumors

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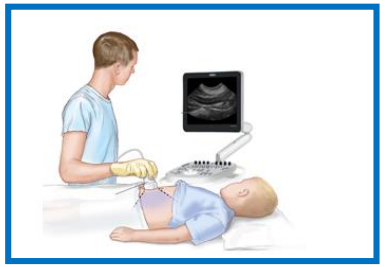
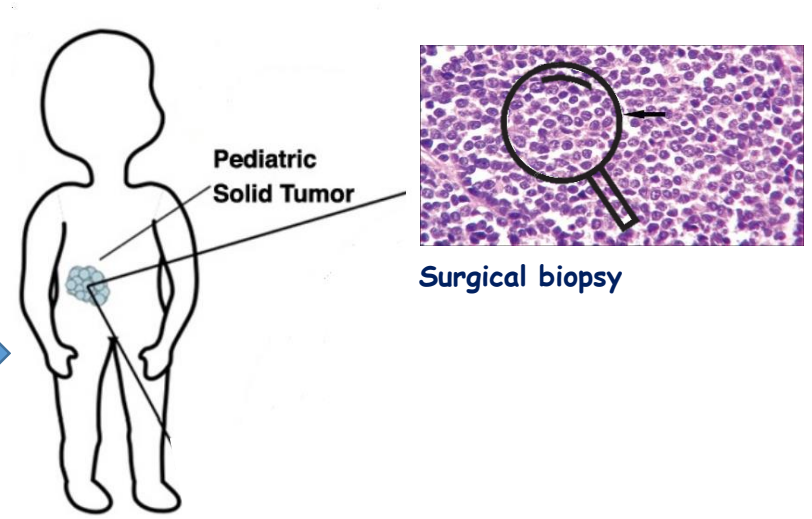
Detailed clinical history AND
complete physical examination

Laboratory studies



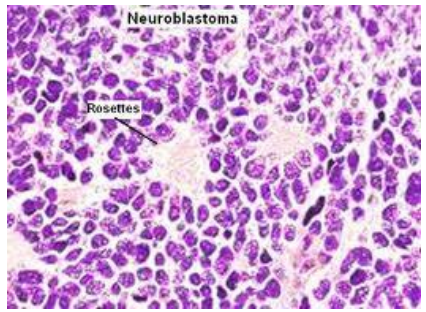
Imaging studies



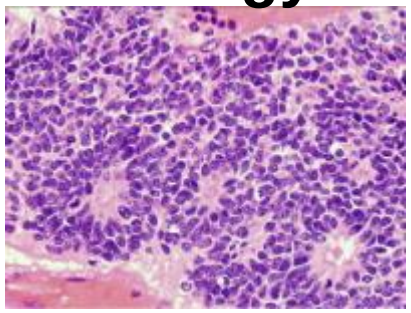
Surgical biopsy

Conventional diagnosis in pediatric solid tumors

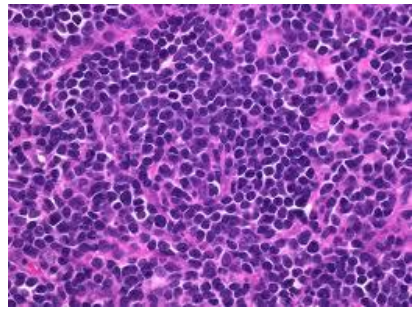
Histology:



Neuroblastoma

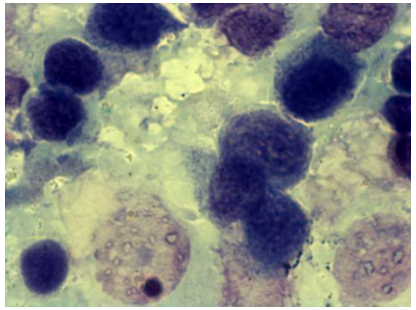


Primitive Neuroectodermic tumor - PNET

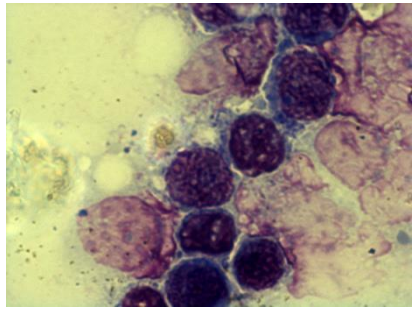


Non-Hodgkin lymphoma

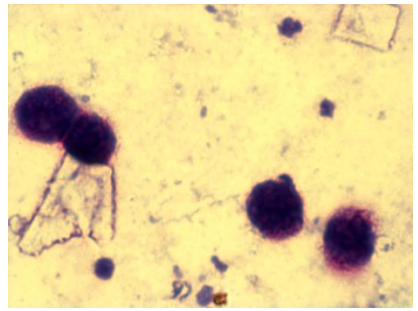
Cytology:



Neuroblastoma



PNET



Non-Hodgkin lymphoma

Histopathologic description of these cancer subtypes:

Histology:

IT IS A BLUE SMALL ROUND CELLS TUMOR

Neuroblastoma

PNET

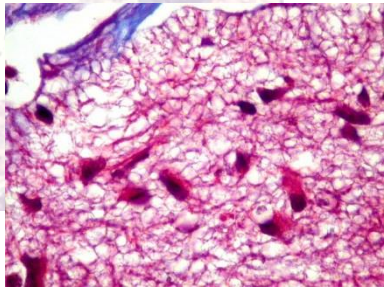
Non-Hodgkin lymphoma

Origin in embryonic tissues

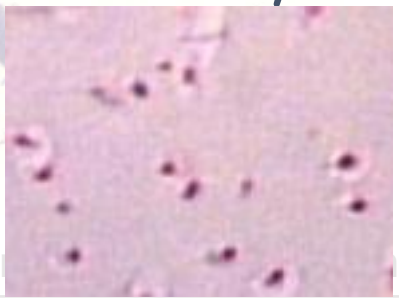
Cytology:

principally

ectoderm



mesenchyme



Clinical suspicion and conventional diagnosis in pediatric solid tumors

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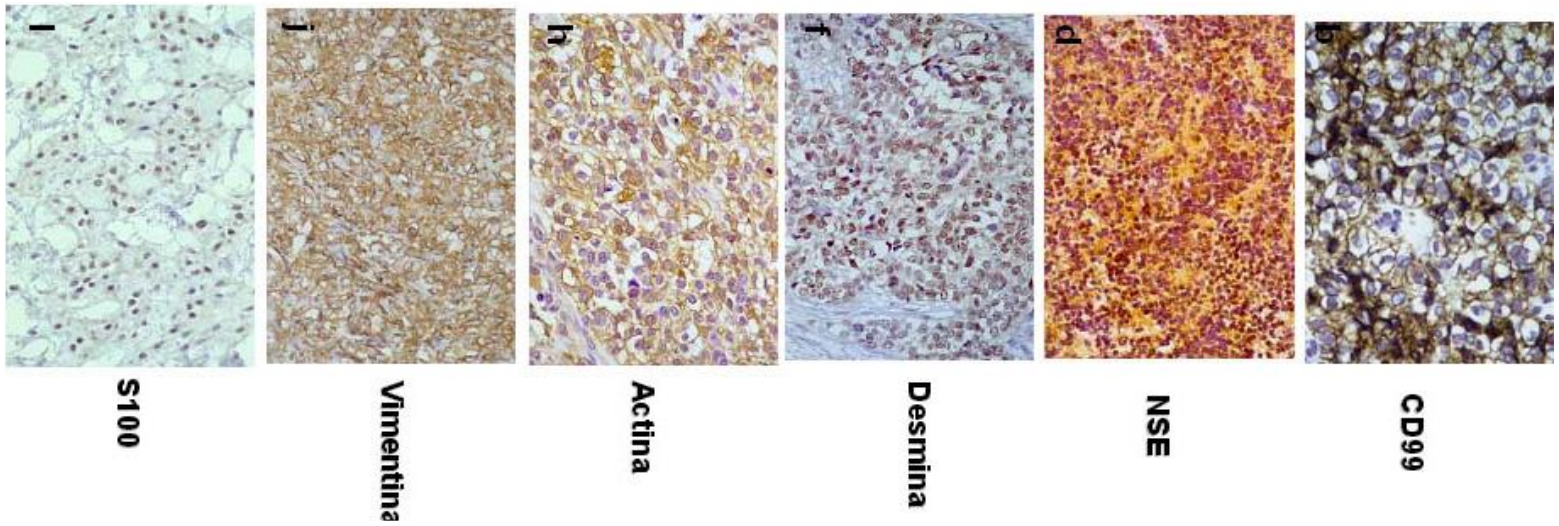
- Tumors from embryonic origin
- Fast growth
- Clinical manifestations simulate benign conditions of children
- Diagnostic is mostly based in histopathology/ immunohistochemistry



Histopathology plus immunohistochemistry *vs.* Flow Cytometry

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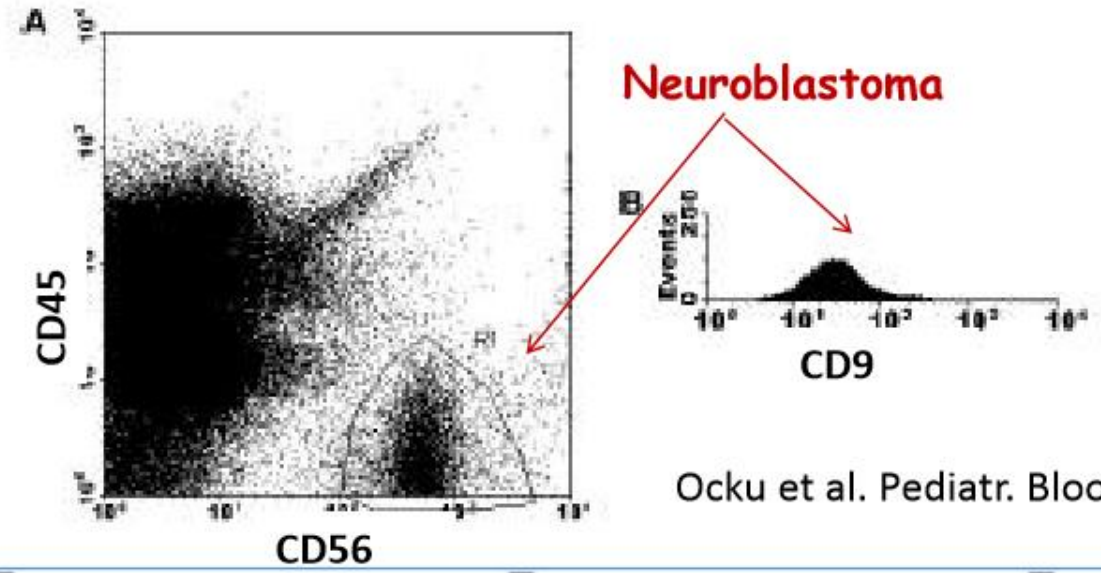
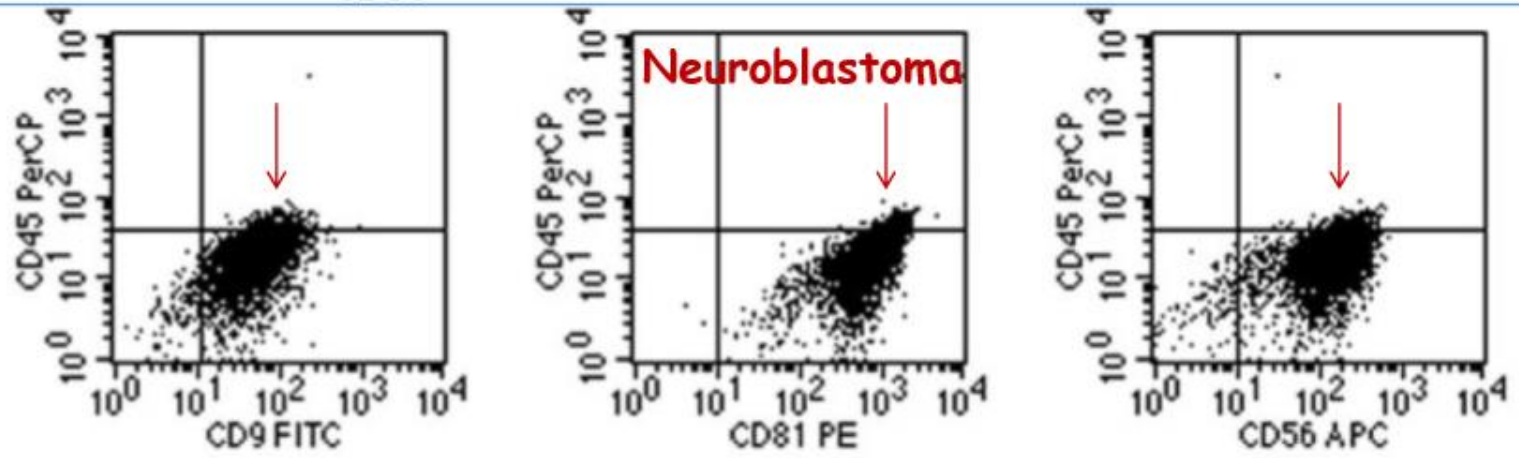
	Histopathology and Immunohistochemistry	Flow Cytometry
Time to diagnostic	8 – 15 days	**Few hours**
Tumor organization	**Tissue organization**	Need tumor disaggregation
Sample storage	**Tissue conservation**	Need cell viability
Markers	One each study	**Several per study (≥8)**
Less represented subpopulations	No identified	**Identified**
Antibody costs	Higher	**Lower**
Diagnostic Criteria	**Established**	On going
Experience	**Higher**	Lower, but **automated analysis** is possible

First studies using Flow Cytometry in pediatric solid tumors

Bone Marrow



Ocku et al. Pediatr. Blood Cancer. 2005; 45: 787-



Swerts et al. Clinical Cytometry. 2004; 61B:9-19

PLOS ONE

PUBLISH ABOUT BROWSE SEARCH

advanced search

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

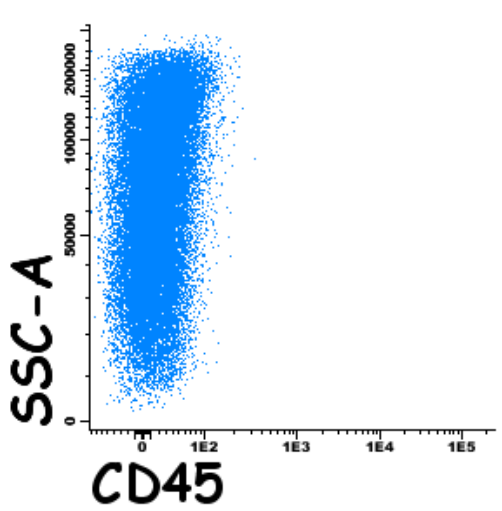
Contribution of Multiparameter Flow Cytometry Immunophenotyping to the Diagnostic Screening and Classification of Pediatric Cancer

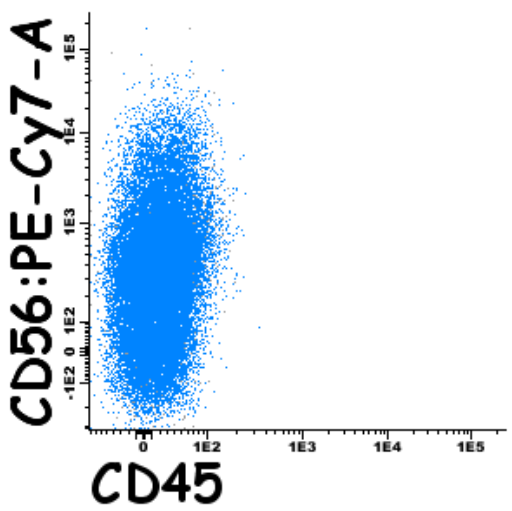
Cristiane S. Ferreira-Facio, Cristiane Milito, Vitor Botafogo, Marcela Fontana, Leandro S. Thiago, Elen Oliveira, Ariovaldo S. da Rocha-Filho, Fernando Werneck, Danielle N. Forny, Samuel Dekermacher, Ana Paula de Azambuja, Sima Esther Ferman, Paulo Antônio Silvestre de Faria, [...], Elaine S. Costa [view all]

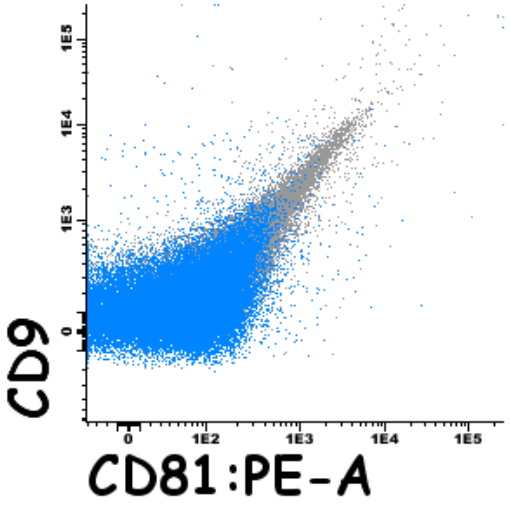
Published: March 5, 2013 • https://doi.org/10.1371/journal.pone.0055534

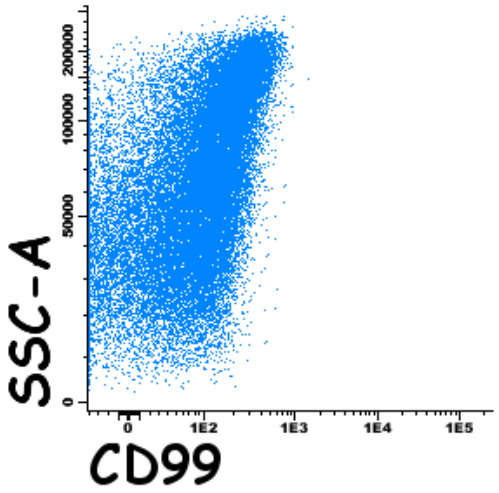
Ferreira-Facio et al. PLOSone, 2013 8(3):e55534

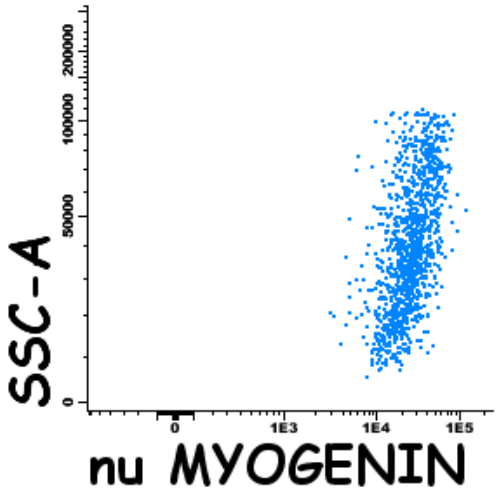
Immunophenotypic profile of a Rabdomyossarcoma

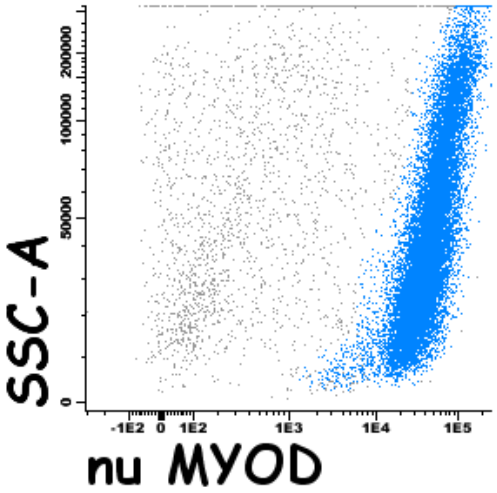












Solid tumor processing

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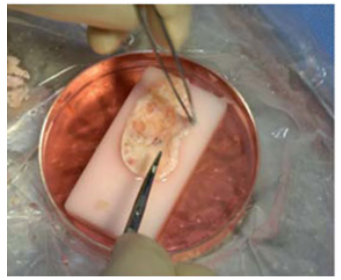
Samples collected in chirurgic center and processed < 6h

PBS
4°C

Weight/ measures/ registration

Imprint slides

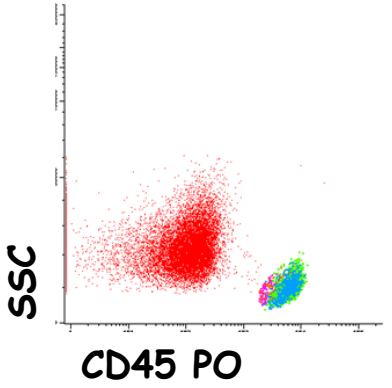


Mechanic dissociation

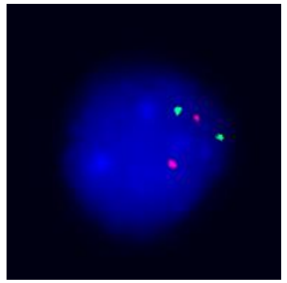
PBS Albumin 0.5%
Filtration (200 µm)

Cells in suspension freshly processed

Multiparametric Flow cytometry



FISH

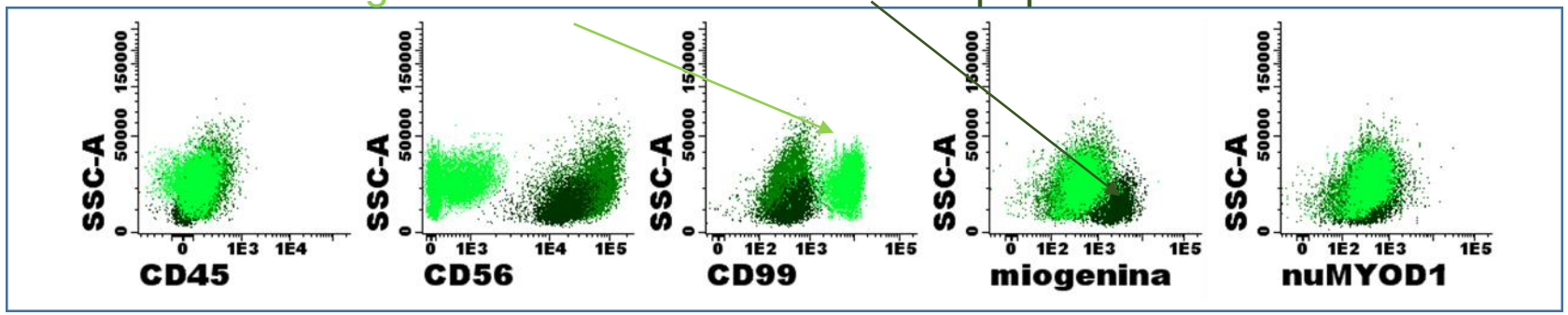


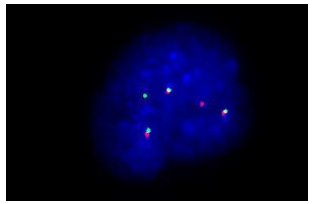
Freezing of viable cells (BFS + DMSO10%)

Integrated analysis

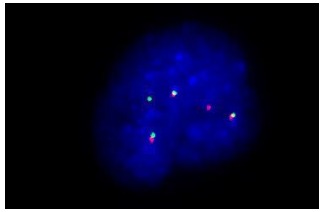
MFC, cell cycle and FISH

Ewing sarcoma but with a RMS subpopulation

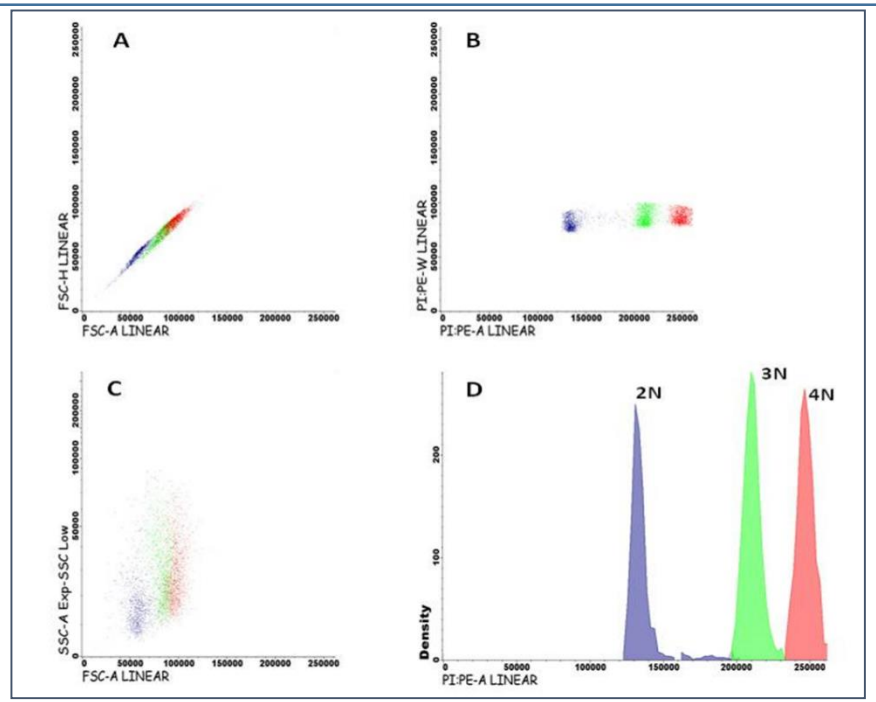


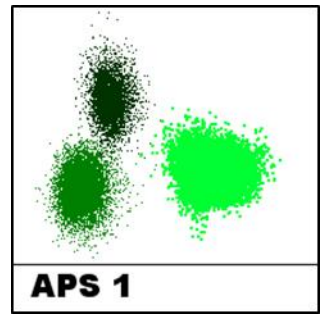


22q12"break apart"



Pax3 t(2;13)





OUR FIRST PUBLISHED RESULTS

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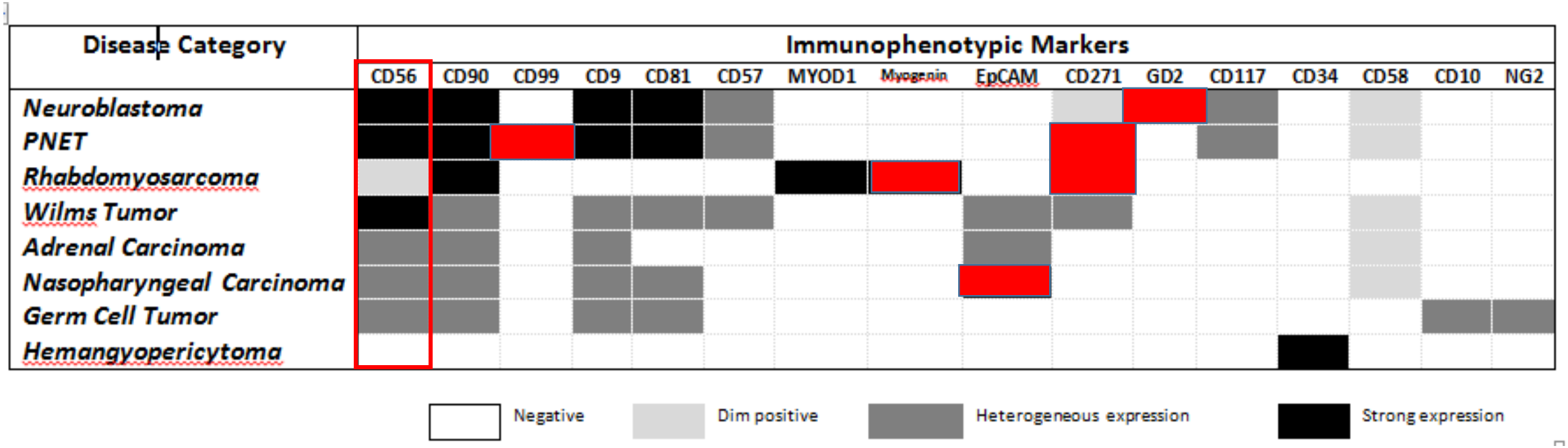


Ferreira-Facio et al. PLOSone, 2013 8(3):e55534

The first phase of pediatric solid tumors project was dedicated to

Development of an 8-color/12 markers combination single tube for

the diagnostic screening of tumor cells

& immune response monitoring

in samples with suspicion of Pediatric Tumors

PHASE 1 -SCREENING

PHASE 1. SCREENING TUBES (103 samples)

FITC	PerCPCy5	PE	PE-cy7	APC	APC-C750
CD8+smIgλ	CD4+CD19	CD56+smIgκ	CD56	CD3	CD45
UCHT-4+policlonal	SK3+HIB19	C5.9+policlonal	Clone 11A9		HI30

LymphoclonalTM
(adapted with CD56 and CD45)

Pacific Blue	OC515	FITC	PerCPCy5	PE	PE-cy7	APC	APC-C750
cyCD3	CD45	cyMPO	CD34	cyCD79a	CD19	CD7	smCD3
UCHT-1	GA90	2C7	581	HM57	19-1	HULY-M2	UCHT-1

ALOTTM

Identification of hematopoietic/reactive normal cells

Neutrophils: $CD45^+/MPO^+$
Monocytes: $CD45^+/MPO^+/CD4^+$
T lymphocytes: $CD45^+/smCD3^+/cyCD3^+/CD7^+$
B lymphocytes: $CD45^+/CD19^+/cyCD79a^+/\ CD20^+$
NK cells: $CD45^+/CD7^+/CD56^+$

Identification of non-hematopoietic neoplastic cells

$CD45^-/CD56^+$ or $CD45^-/CD56^{++}$ non-hematopoietic pediatric solid tumor

PHASE 1 - SCREENING

PHASE 1. SCREENING TUBES (103 samples)

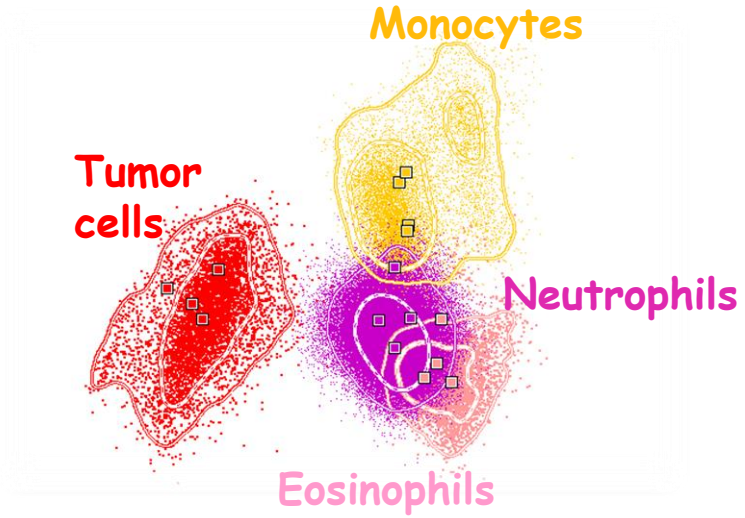
Identification of normal vs neoplastic cells (sequential steps):

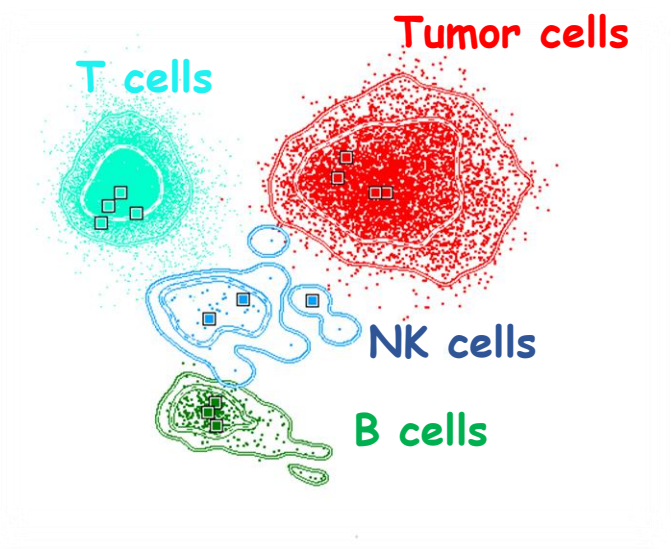
Pacific Blue	OC515	FITC	PerCPCy5	PE	PE-cy7	APC	APC-C750
CD20+CD4	CD45	CD8+smIgλ	CD5	CD56+smIgκ	CD19+TCRδ	smCD3	CD38
2H7+RPA-T4	GA90	UCHT-4+policlonal	UCHT-2	C5.9+policlonal	19-1+TCR-1	UCHT-1	LD38

LST™

Pacific Blue	OC515	FITC	PerCPCy5	PE	PE-cy7	APC	APC-C750
cyCD3	CD45	cyMPO	CD34	cyCD79a	CD19	CD7	smCD3
UCHT-1	GA90	2C7	581	HM57	19-1	HULY-M2	UCHT-1

ALOT™





PHASE 2 CHARACTERIZATION

PHASE 1. Characterization panel of pediatric solid tumor (91 samples)

PO	FITC	PE	PERCP Cy5.5	PE Cy7	APC	APC-H7
CD45	**CD57**	**CD90**	**CD34**	**CD56**	**Epcam**	
HI30	HNK-1	5.00E+10	8G12	N901/NKH1	EBA-1	
Invitrogen	BD Biosciences	BD Biosciences	BD Biosciences	Beckman Coulter	BD Biosciences	
CD45	**CD99**	**CD81**	**CD9**	**CD56**	**CD117**	
HI30	TÜ12	JS-81	M-L13	N901/NKH1	104D2	
Invitrogen	BD Biosciences	BD Biosciences	BD Biosciences	Beckman Coulter	BD Biosciences	
CD45	**CD58**	**CD38**		**CD56**	**CD10**	
HI30	1C3	HB-7		N901/NKH1	HI10A	
Invitrogen	BDBioscience	BD Biosciences		Beckman Coulter	BD Biosciences	
CD45		**CD271**		**CD56**		
HI30		C40-1457		N901/NKH1		
Invitrogen		BD Biosciences		Beckman Coulter		
and 4 single tubes with the following antibodies + rabbit anti-IgG FITC						
	$_{Nu}$MYOD	**$_{Nu}$Myogenin**	**GD2**	**$_{Cy}$Desmina**		
	5.8^{a}	F5D	14.G2a	RD301		
	BD Biosciences	BD Biosciences	BDBiosciences	BD Biosciences		

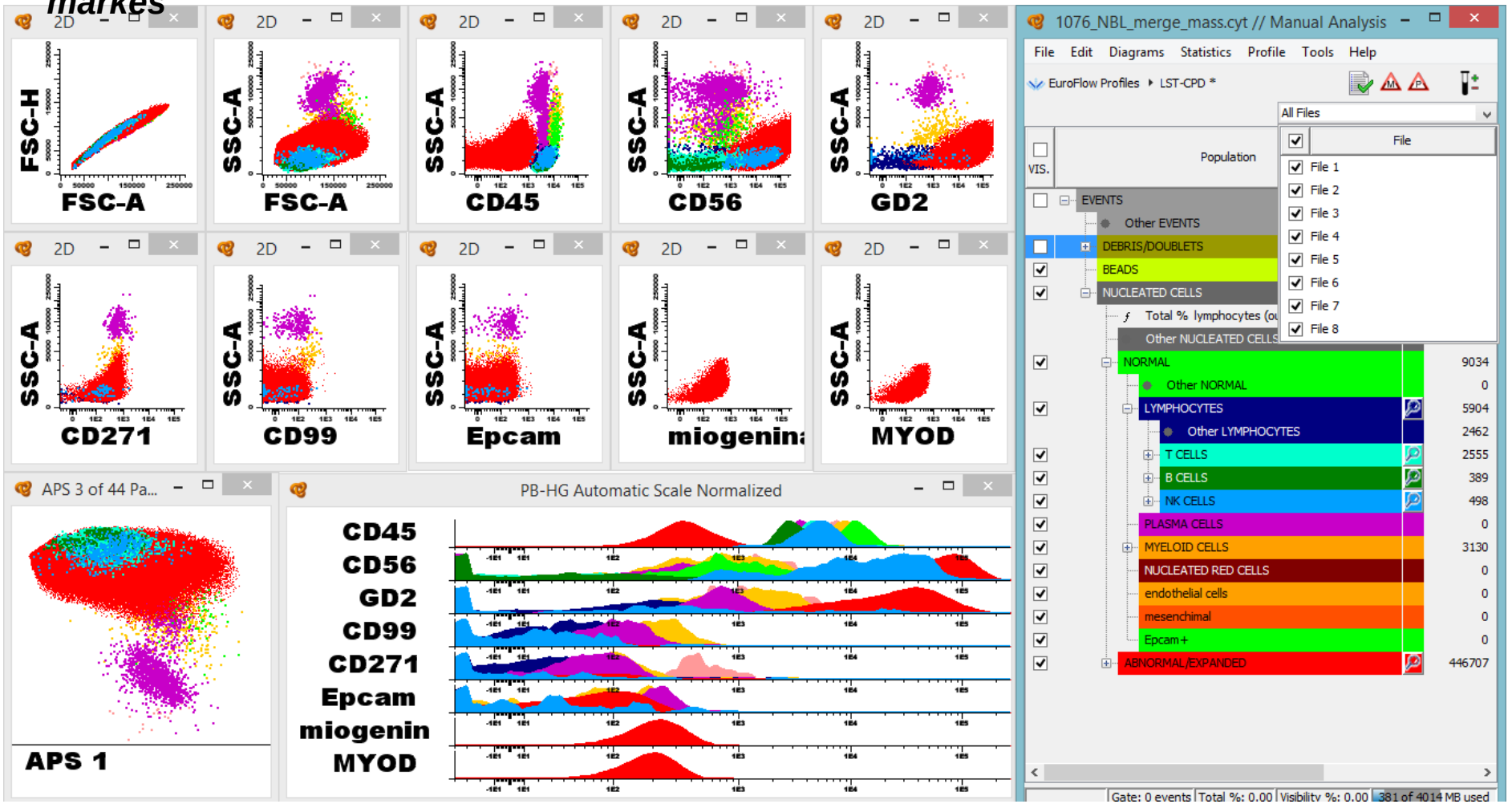
PHASE 2 CHARACTERIZATION

PHASE 2. Characterization panel of pediatric solid tumor (53 samples)

PO	BV421	FITC	PE	PERCP Cy5.5	PE Cy7	APC	APC-H7
CD45		CD99	CD71	CD9	CD56	GD2	CD81
HI30		HNK1	C401457	M-L13	N901	14.2Ga	JS-81
CD45		CD58	CD90	CD34	CD56	CD90	CD38
HI30		1C3	Thy1	L17F12	N901	Thy1	HB7
CD45	CD271	CD44	CD99	CD9	CD56	Epcam	
HI30	C401457	L178	JS-81	M-L13	N901	EBA-1	
CD45		nuMYOD	nuMyogenin	Epcam	CD56	CD10	
HI30		5.8[a]	F5D	EBA-1	N901	HI10A	

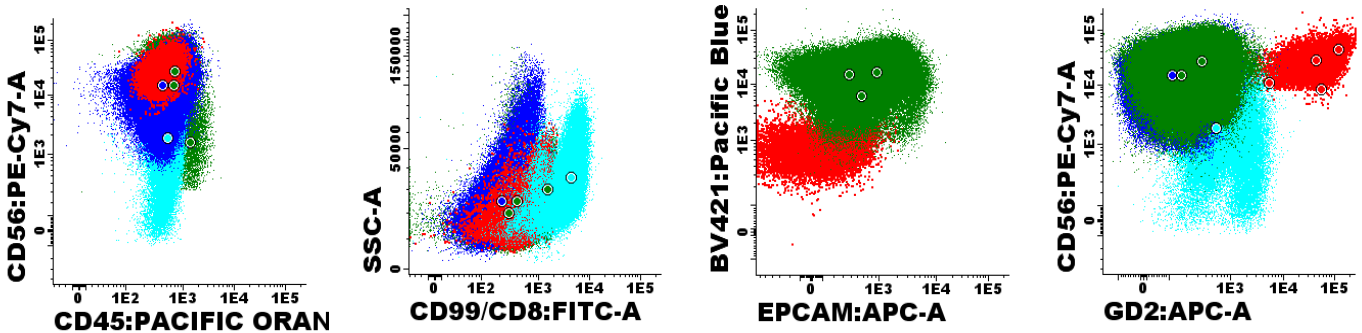
File merge and calculate data analysis

One sample file merged with a total of 45 markes



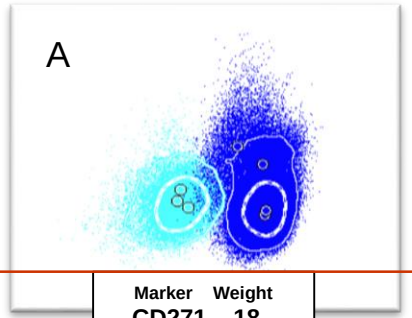
STUDY DESIGN: CLASSIFICATION MARKERS

EuroFlow



PNET *VS* **RMS**

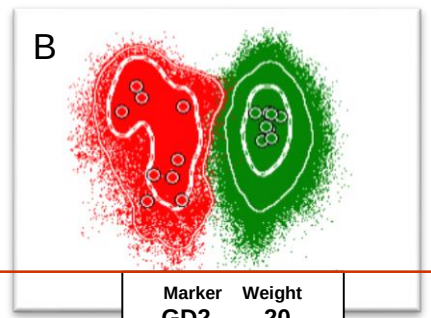
A



Marker	Weight
CD271	18
GD2	14
CD9	13
CD90	8

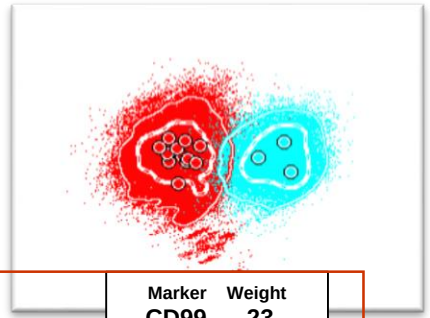
NBL *VS* **WT**

B



Marker	Weight
GD2	20
EPCAM	12
CD56	11
CD81	8

NBL *VS* **PNET**



Marker	Weight
CD99	23
GD2	16
CD56	9
CD90	6

8-COLOR STOT tube

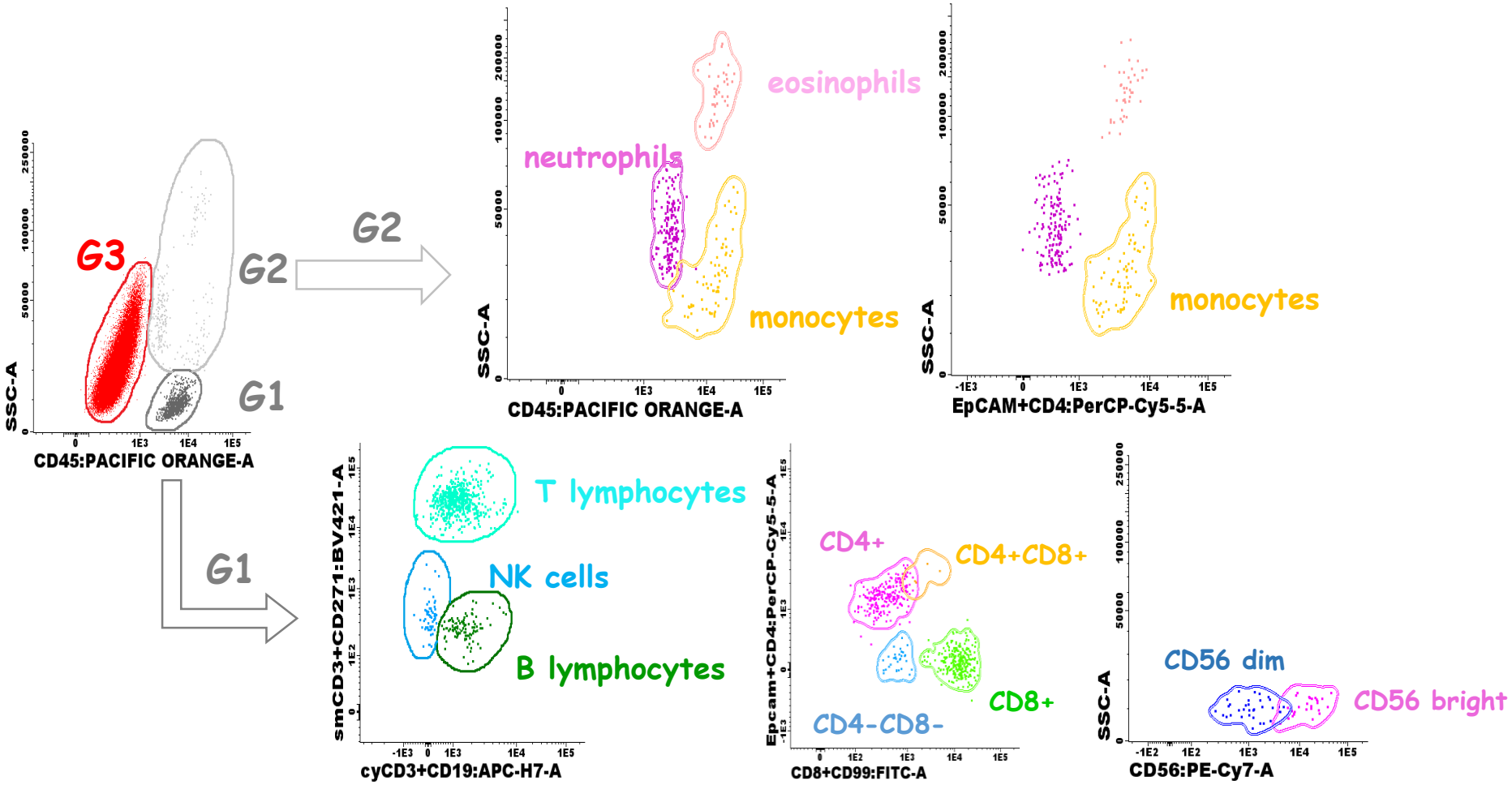
EuroFlow
a division of ESLHO

BV421	PO	FITC	PE	PERCP Cy5.5	PE Cy7	APC	APC-H7
cyCD3+CD271	CD45	CD99+CD8	nuMyogenin	Epcam+CD4	CD56	GD2	smCD3+CD19
UCHT1+C40-1457	HI30	Tü12+UCH-T4	F5D	SK3+EBA-1	N901	14.G2a	SK7+SJ25C1
BDBiosciences	Invitrogen	BDBiosciences	BDBiosciences	cytognos/ BD Biosciences	BeckmanCoulter	BDBiosciences	BDBiosciences+ BeckmanCoulter

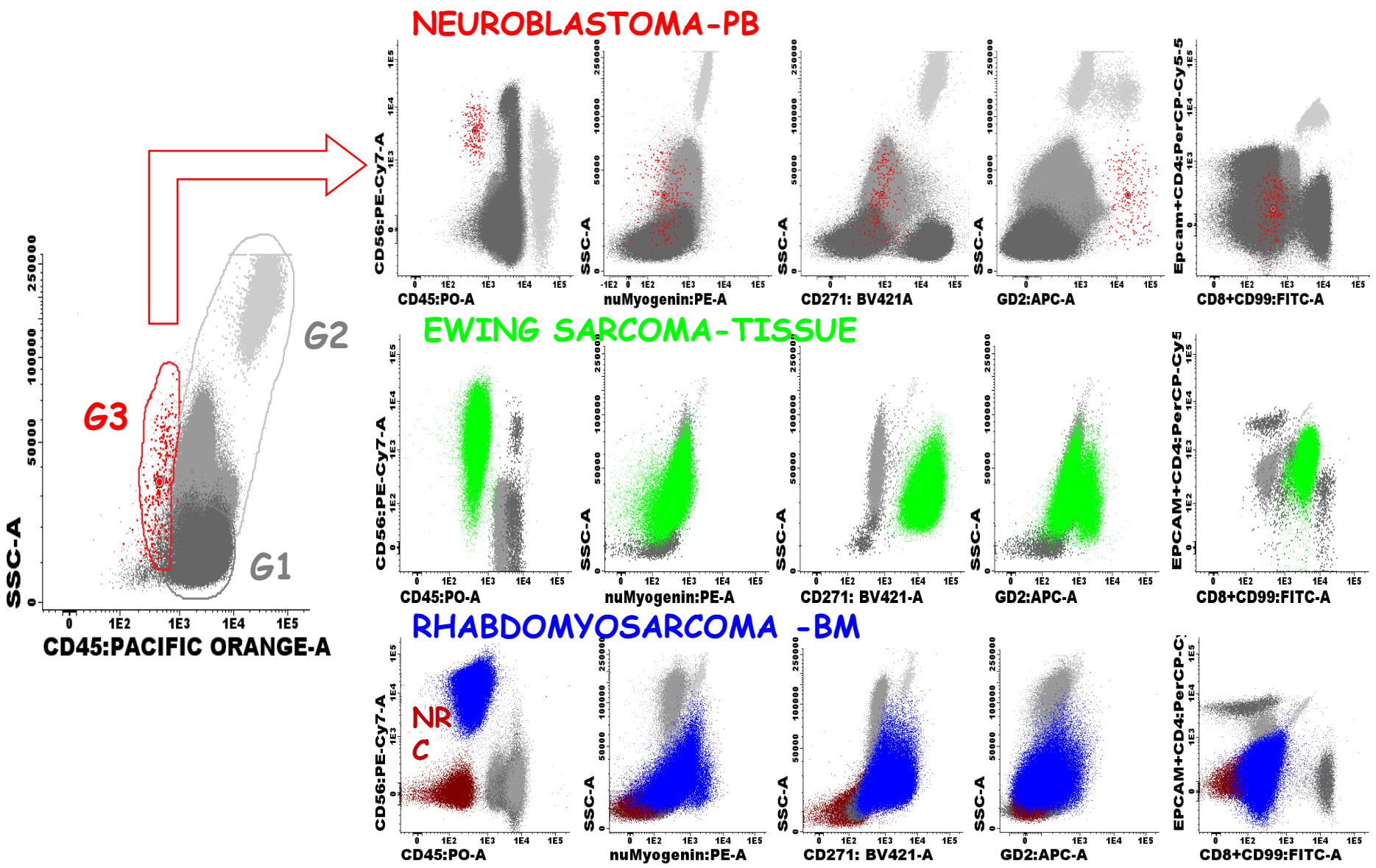
an 8-color/12 markers combination single tube for the diagnostic screening of tumor cells

& immune response monitoring in samples with suspicion of Pediatric Tumors

STOT: Gating Strategy Analysis



STOT: Gating Strategy Analysis



NRC: nucleated red cells; BM: bone marrow; PB: peripheral blood

STOT markers in most frequent non hematopoietic tumors

Tumor	GD2	CD99	Miogenin	EpCAM	CD271	CD56	CD45
Neuroblastoma tumors	POS	NEG	NEG	NEG	NEG	POS	NEG
Extraosseus Ewing Sarcoma (PNET)	LO	POS	NEG	NEG	POS	POS	NEG
Rhabdomyossarcoma	NEG	NEG	POS	NEG	HI POS	POS	NEG
Osteosarcoma	NEG	NEG	NEG	NEG	HI POS	POS	NEG
Wilms Tumor	NEG	NEG	NEG	POS	HI POS	POS	NEG
Clear cell sarcoma	POS	NEG	NEG	POS	HI POS	POS	NEG
Germ cells tumor	NEG	NEG	NEG	NEG	Variable	Variable	NEG
Nasopharyngeal carcinoma	NEG	NEG	NEG	POS	NEG	POS	NEG

CONCORDANCE ANALYSIS HISTOPATHOLOGY VS CYTOMETRY

EuroFlow

A total of 296/350 samples (84.5%) were evaluated (Kappa coefficient)
The observed agreement was **93,9%** (278/296 samples)

HISTOPATHOLOGY / CYTOMETRY	DISEASE FREE	REACTIVE	WHO CLASSIFICATION	NON-HEMATOPOIETIC	NO DIAGNOSIS
disease free	77	0	2	0	0
reactive	0	57		0	0
who classification	0	0	122	22	16*
no diagnosis	0	0	0	0	0

Neuroblastoma, Rhabdomyosarcoma,
Wilms Tumors, Ewing Sarcoma,
non Hodgkin Lymphoma

CONCORDANCE ANALYSIS HISTOPATHOLOGY VS CYTOMETRY

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Rare pediatric solid tumors,
Germ cells tumors,
Low cellularity samples

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Half of the samples infiltrated by Hodgkin Lymphoma
Low cellularity/ low viability samples

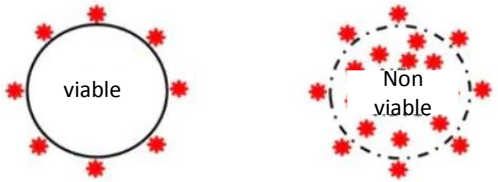
Next Steps: Viability dyes for pediatric solid tumors

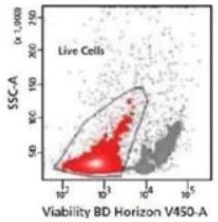
Aim: Test of viability dyes in order to a better discrimination between viable and nonviable cells

Fixable Viability Stain 780

Product Information

Material Number:	**565388**
Size:	200 µg





https://www.bdbiosciences.com/

8-COLOR STOT tube

EuroFlow
a division of ESLHO

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BDBiosciences	Invitrogen	BDBiosciences	BDBiosciences	cytognos/ BD Biosciences	BeckmanCoulter	BDBiosciences	BDBiosciences+ BeckmanCoulter

an 8-color/12 markers combination single tube for the diagnostic screening of tumor cells

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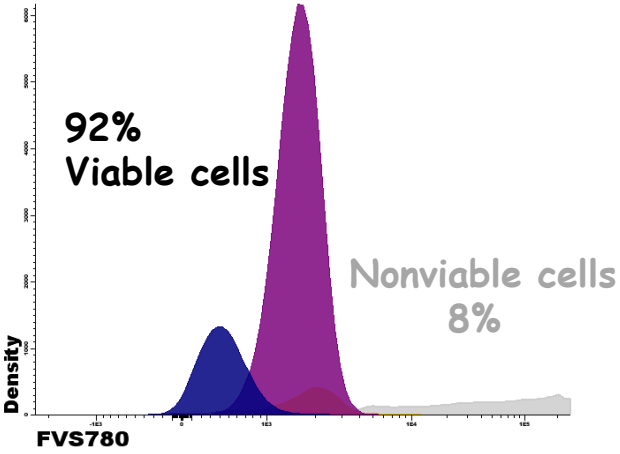
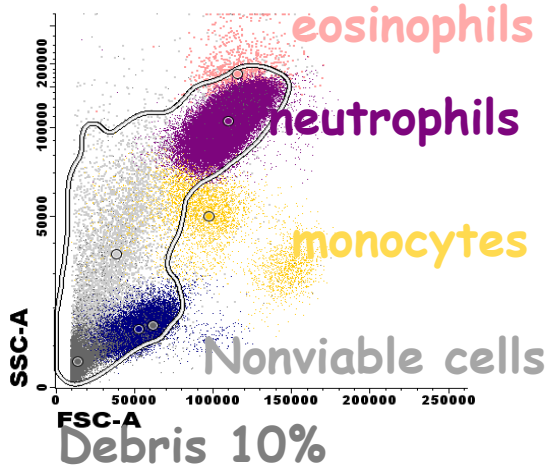
Next Steps: Viability dye for pediatric solid tumors

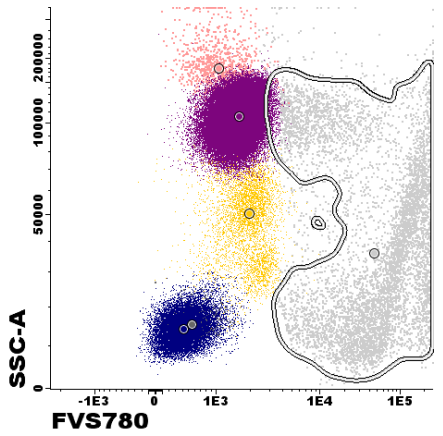
EuroFlow

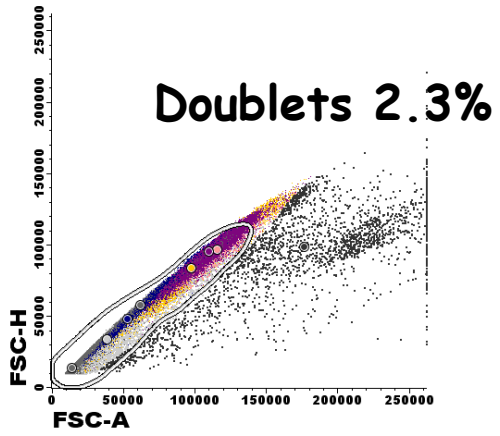
- Peripheral blood experiment

FVS780 1µl/1000 µl (datasheet recomendation)

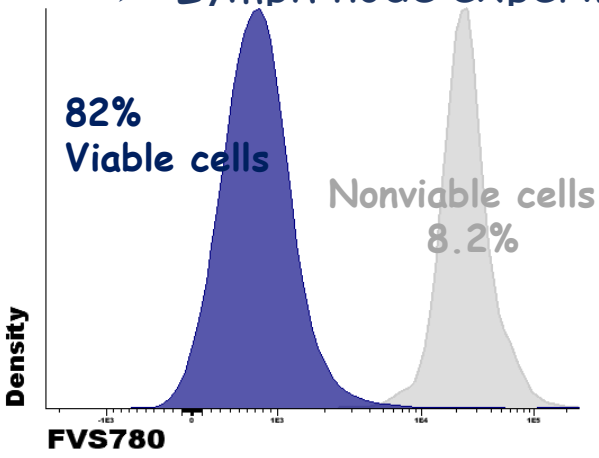


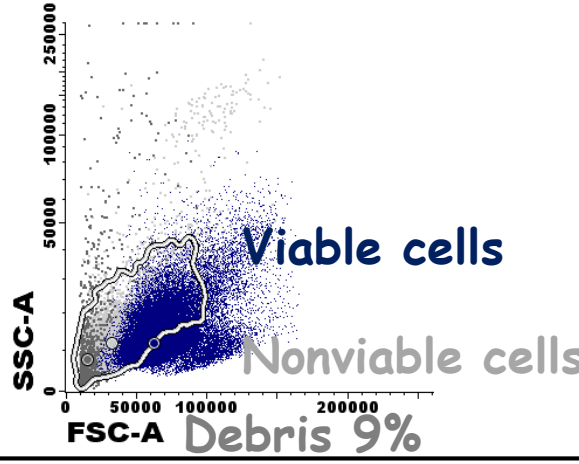


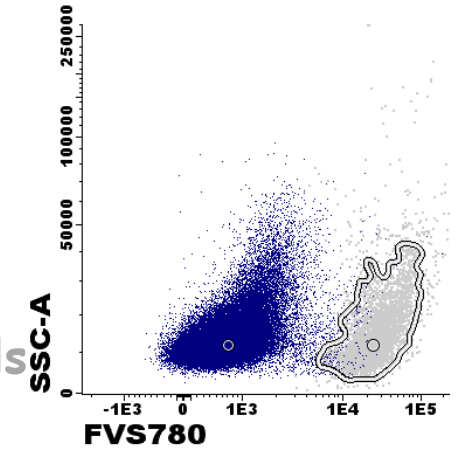


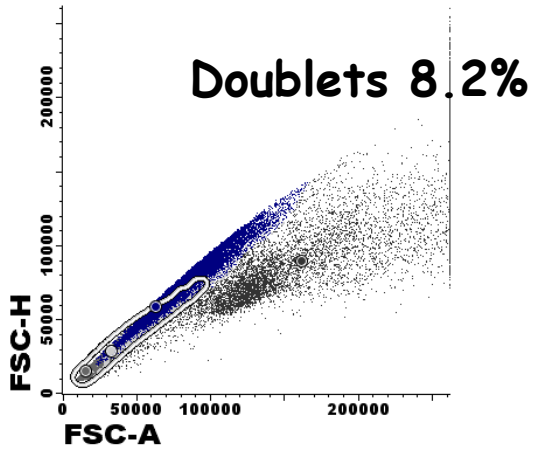


- Lymph node experiment







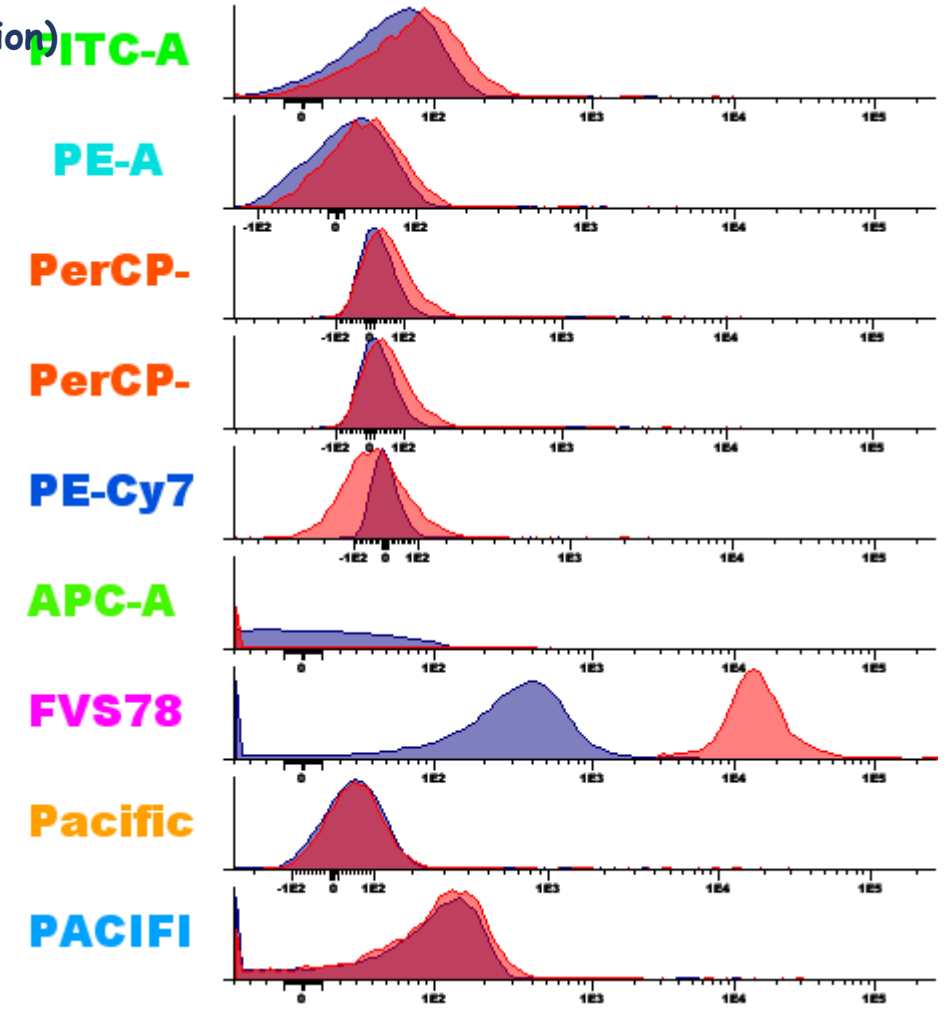


Next Steps: Viability dyes for pediatric solid tumors

EuroFlow

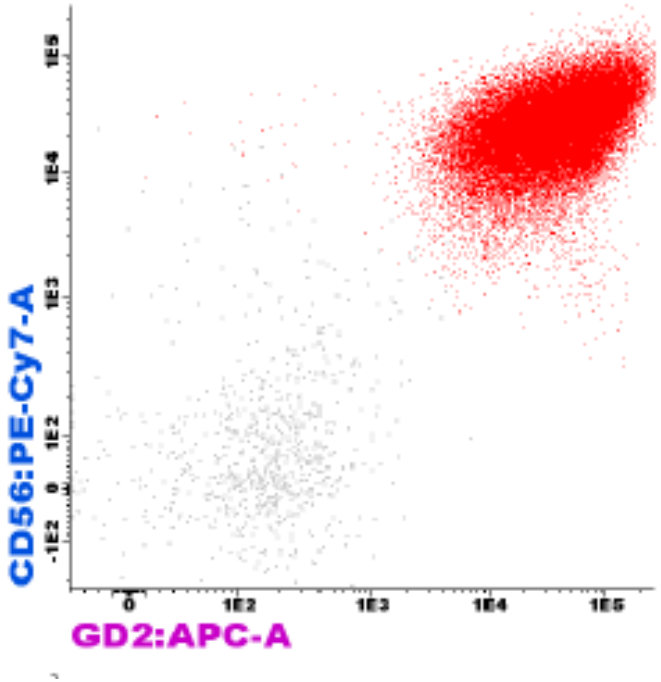
- Lymph node experiment

FVS780 1µl/1000 µl (datasheet recomendation)

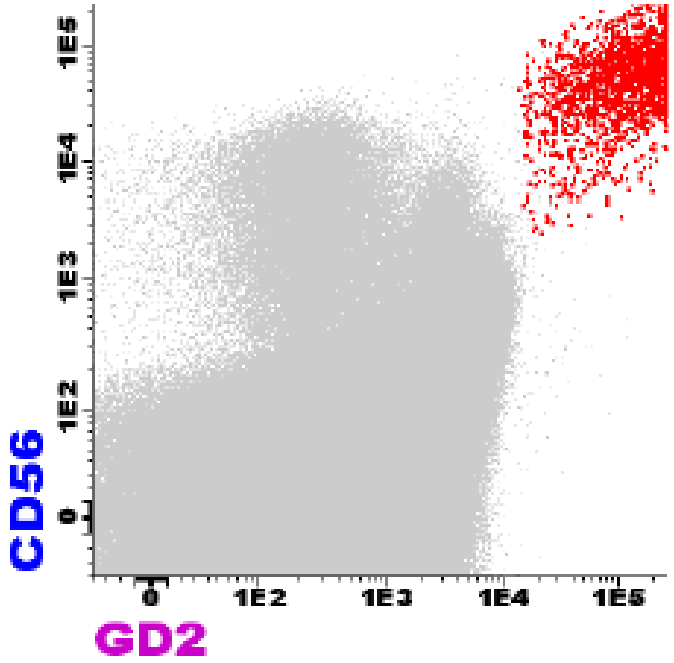


STOT-APPLICABILITY – CTC AND MTC DETECTION

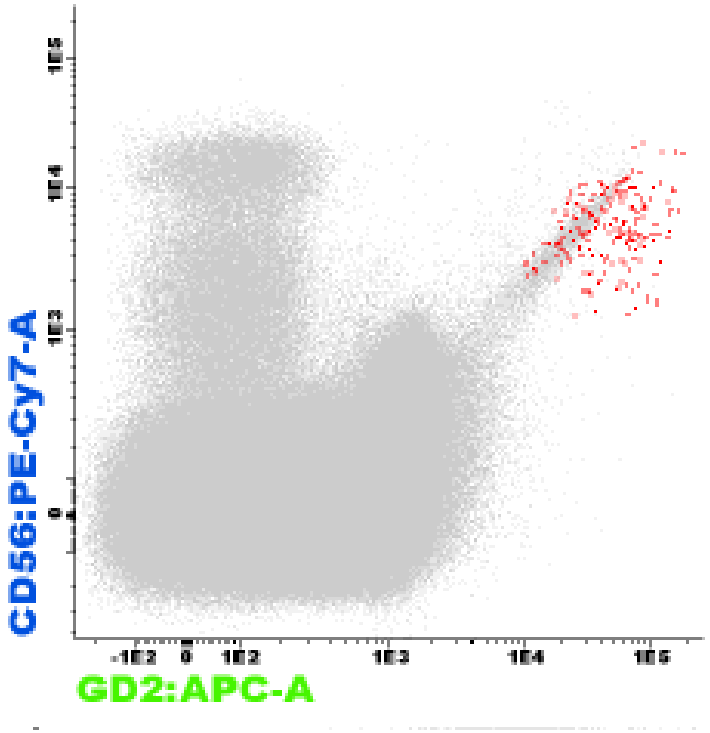
Neuroblastoma



Tumor mass:
98.46% of tumor cells
200.000 Events



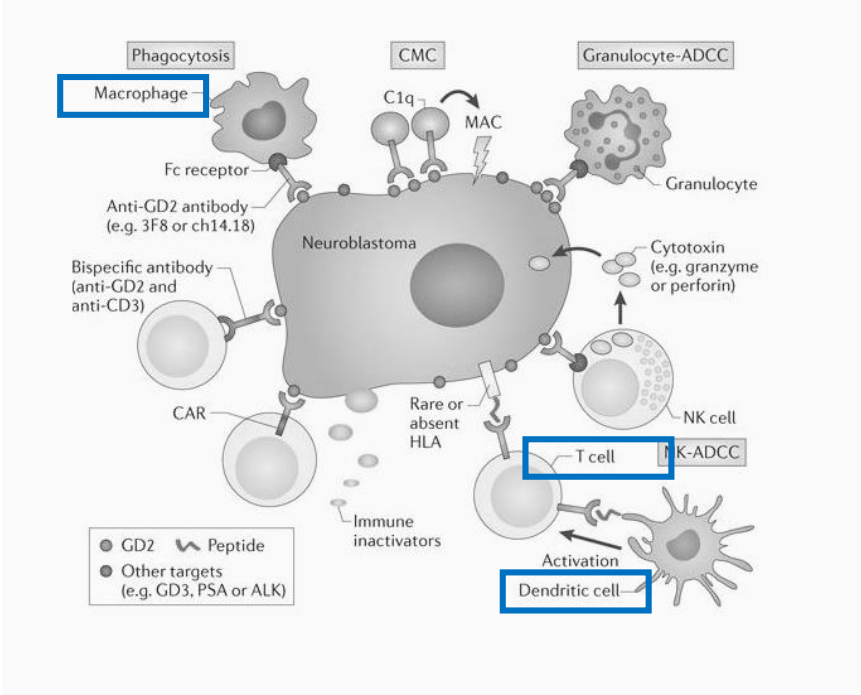
Bone marrow
0,04% of tumor cells
5.000.000 Events



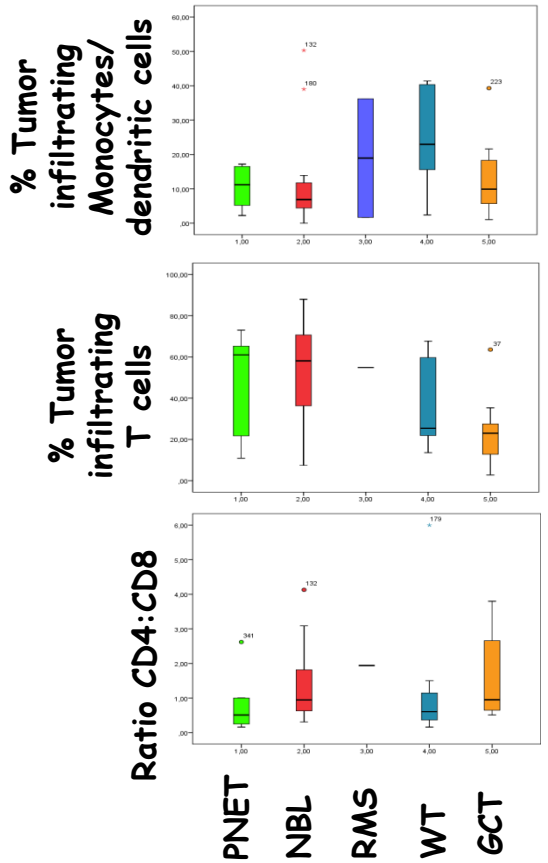
Peripheral blood
0,01% of tumor cells
2.000.000 Events

STOT-APPLICABILITY

Immune monitoring



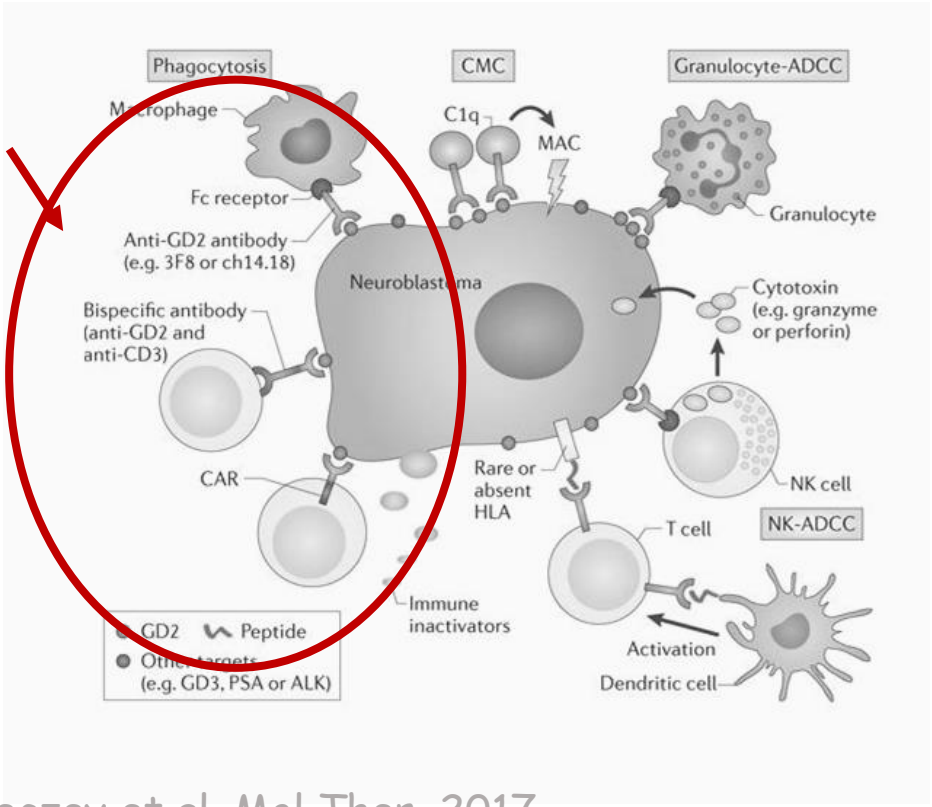
Characterization of immune response on tumor masses



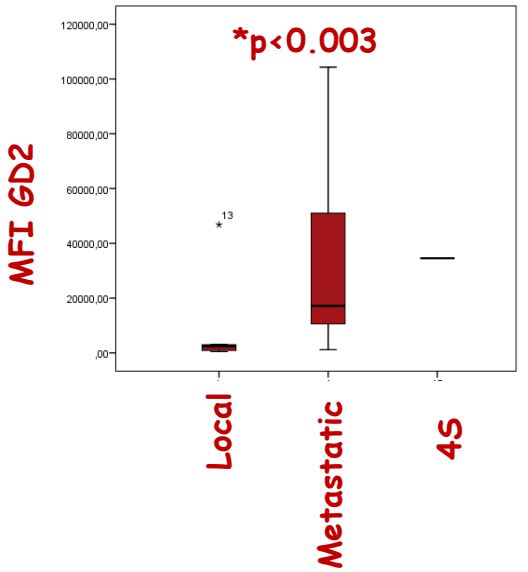
Camisaschi et al. BMC Cancer, 2018
Chowdhury et al. Cancer Immunol Immunother, 2014

STOT-APPLICABILITY

- Orientation of immunotherapies
- Potential prognostic evaluation

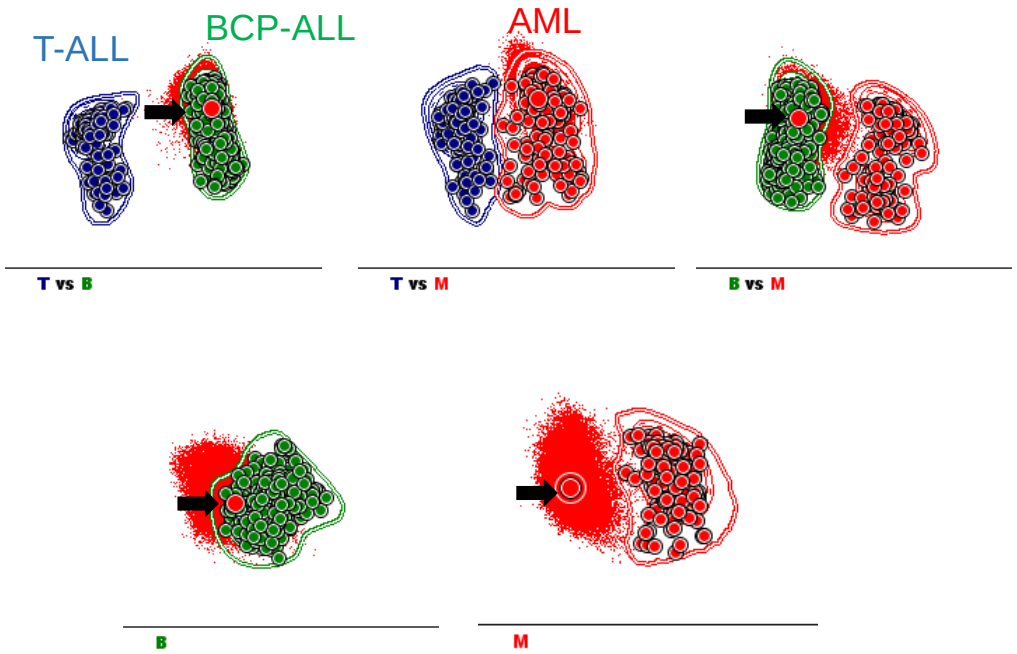


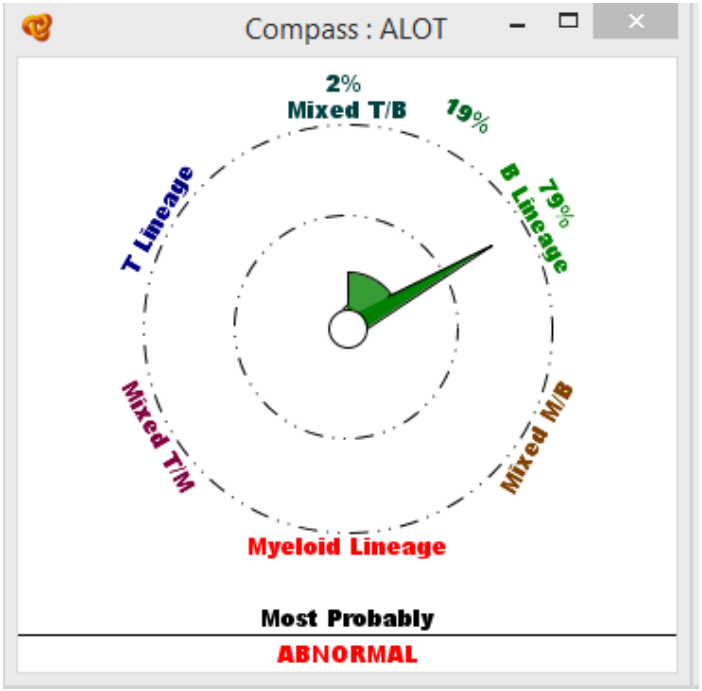
Quantification of protein expression (GD2) on tumor cells



Heczey et al. Mol Ther, 2017
Federico et al. Clin Cancer Res, 2017
Le et al. Frontiers Immunol, 2017

NEXT STEPS: TO BUILD A DATABASIS THAT ALLOW A AUTOMATED ANALISIS AND A COMPASS FOR DIAGNOSTIC ORIENTATION





EXAMPLE OF ACUTE LEUKEMIAS

Thank you! Obrigado!

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cito.ufrj@gmail.com