Issues on quality aspects applied to off-line Extra Corporeal Photopheresis (ECP).
From our center experience.

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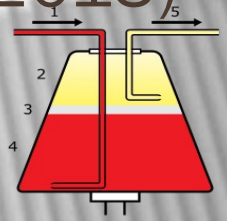
OVERVIEW OF OUR ECP PRACTICE

Off-line ECP since 1998 (ANSM agreement 2013)

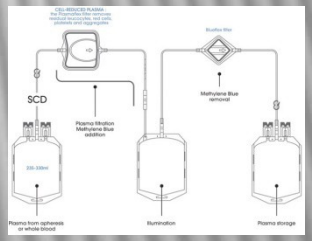


- MNC cytapheresis :

Cell separators: Terumo Spectra ® & Optia ®, Fresenius Comtec ®

1,5 blood volume processed – targeted haematocrit (2-3%)

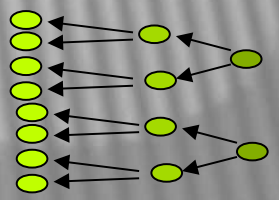
ACD ratio 1/12 -1/10 - Calcium IV infusion



- Cell Therapy Laboratory

volume/haematocrit adjustment

MOP injection ([C] 200 ng/ml) – UV (2 J/cm2) Macopharma®



Quality controls: BCC at reception , BCC & bacteriology on final product

Hospital Stay: ≈ 3h1/2

lymphoproliferation test by CFSE method

CFSE incorporates nucleus in phase M of cell division

CFSE becomes fluorescent when cleaved by cell esterases

Fluorescence fades through cell proliferation

Reading by cytometry at D4 after PHA and OKT3 stimulation

Results expressed by the Remaining Proliferation Index (RPI) :

% (cell CFSE dim) stimulated cells - % (cell CFSE dim) non stimulated cells

Result « efficient » if CFSE < 20 % (proliferation after UV)

Test is invalid if proliferation < 10 % prior to UV irradiation

CFSE vs 3H Thymidin (PHA)

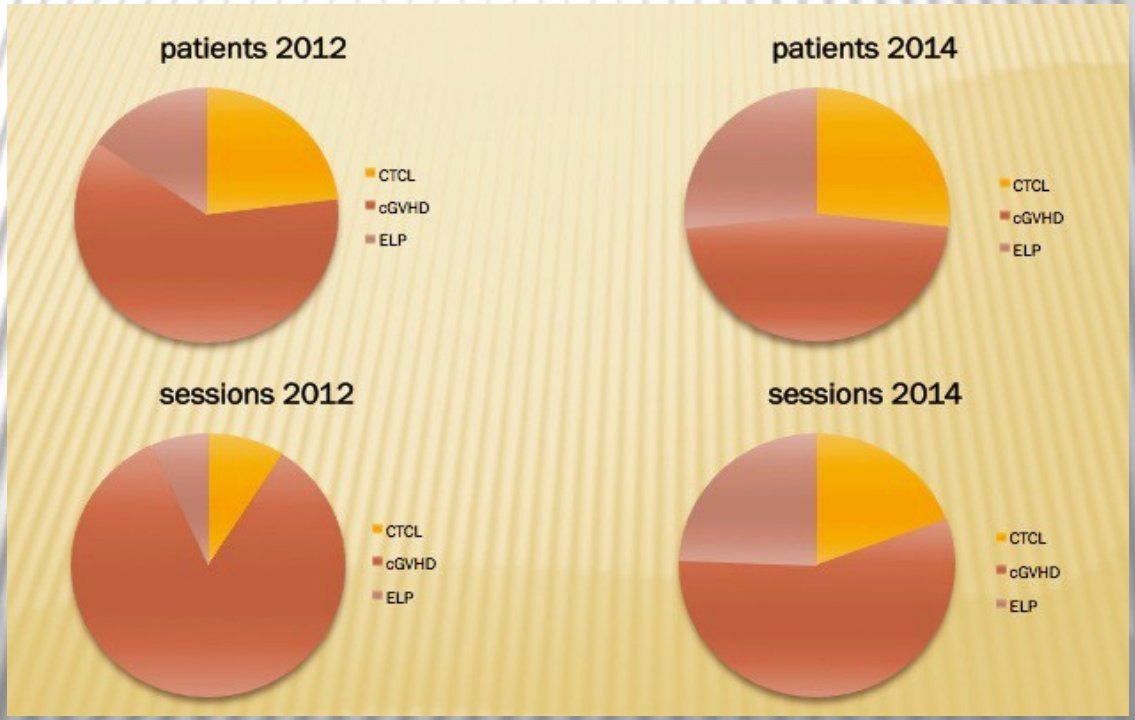
30 healthy subjects

	CFSE dim	3H Thymidin
Median	**70,1%**	**119021 cpm**
	57,3 -78,3	89482-146324
Average	**65,5%**	**117055 cpm**
Standard-deviation	17,8	41314

TREATED PATIENTS

PCE operation 01/01/2012-31/10/2014*		CTCL	cGVHD	ELP	Misc
nb patients	35	5	22	6	2
nb sessions	996	101	752	120	23

2 YEARS EVOLUTION OF OUR INDICATIONS



Quality OF THE MNC HARVEST: OUR RESULTS*

VOLUME MNC	94,4 ± 28,4ml	22 - 252 ml
HAEMATOCRIT	0.85 ± 0.68 % 1/393 out limit, uncontroled	0-4%
Nb WC	6.7 ± 5.9 G	0.6 - 42.5 G
WCC	22.6 ± 19.7 G/L.	2.1 -141.6 G/L.
Monocytes	33,4 ± 12.8 %	4 – 68%
Lymphocytes	58.8 ± 14.9 %	8 – 88 %
PMN	7,4 ± 10,5 %	0 - 53
Platelets	931.3 ± 567.3 G/L.	68 – 2741 G/L.

**Survey on 393 collections (2013-2014)*

Quality OF THE MNC PRODUCT: OUR RESULTS*

- Differences between cell separators

	Platelets (G/L)	PMN (%)
SPECTRA (n=106)	929,1	6,2
OPTIA (n=25)	655,9	9,8
COMTEC (n=56)	1060,7	3,4

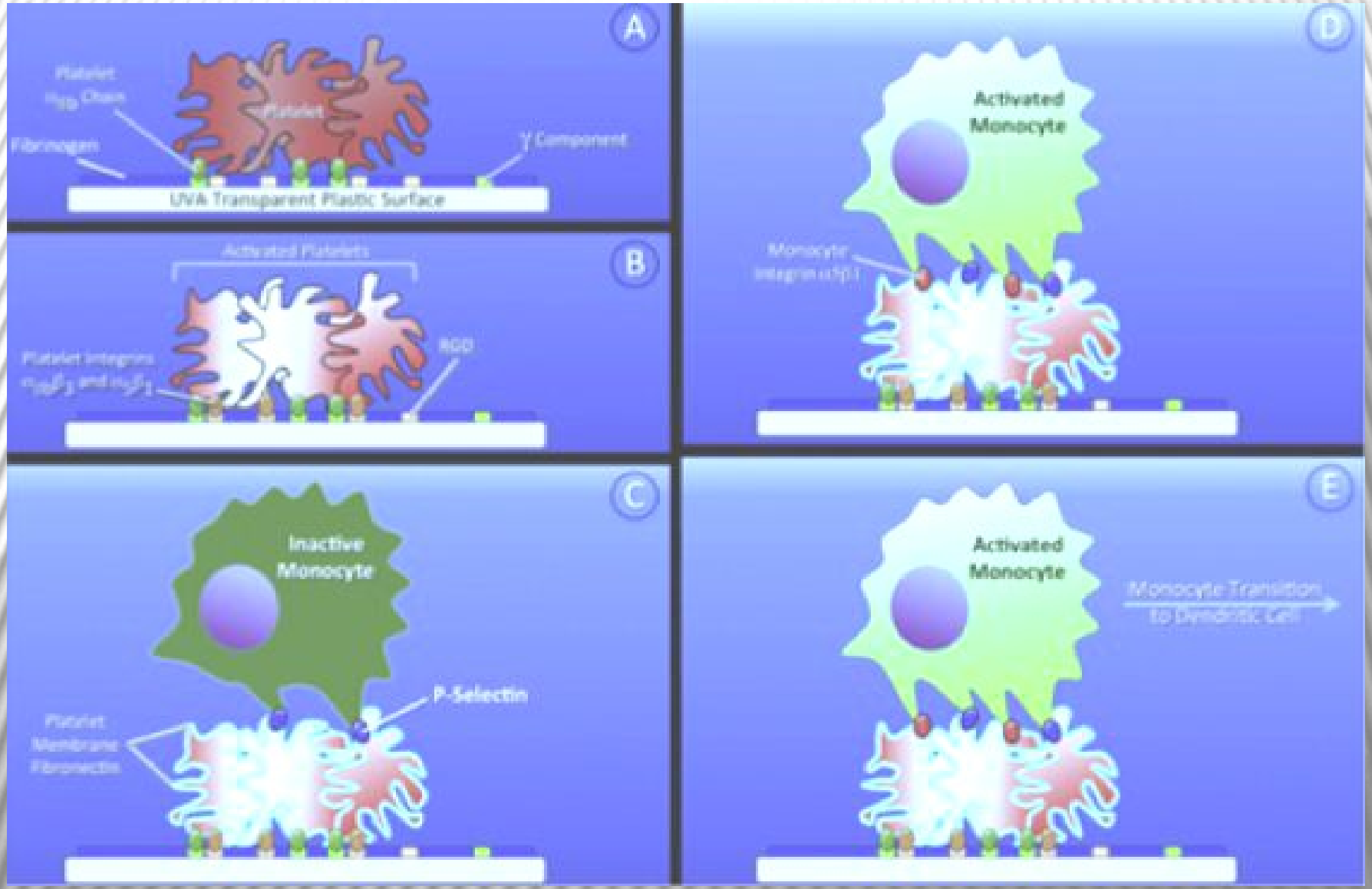
**Survey on 187 collections (2014)*

Quality OF THE MNC PRODUCT: STANDARDS?

Features	Our defined standards	Comment
VOLUME MNC	**none**	Volume is optimized to 300 ml for MOP injection and UV irradiation
HAEMATOCRIT	**≤ 3%**	**Consensus (2% - 3%)***
Nb WC	**none**	**Does Nb influence treatment efficacy?****
Lymphocytes/ Monocytes/PMN	**none**	**Which MNC is important?****
Platelets	**none**	**Aggregates troubling cell manipulation, problem with cytopenic patients**

**Andreu G et al. Transfus Apher Sci. 1994 Dec;15(4):443-54*
Schooneman F. Transfus Apher Sci. 2003 Feb;28(1):51-61

*** Perseghin P et al. Ther Apher Dial. 2007 Apr;11(2):85-93.*



Shear stress 0.5dyne/cm3

Mono → DC: (CD40,CD80, CD83, CCR7)

Edelson RL et al., Transfus Apher Sci 2014

Durazzo et al ., Transfus Apher Sci 2014

Quality OF THE FINAL PRODUCT: results*

Haematocrit	6/393 controls (2 suitable, 4 needed dilution)
Bacteriology control	2 contaminated products

lymphoproliferation tests (CFSE – PHA/OKT3)	RPI invalid: no proliferation before UV radiation	RPI unefficient: proliferation after UV radiation
cGVH (50)	3~	2~
CTCL (13)	1~	0
ELP (2)	1~	0
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New MOP batches (7)	0	0

~ all invalid/unefficient results were rechallenged and not confirmed

**Survey on 393 collections (2013-2014)*

Quality OF THE FINAL PRODUCT: STANDARDS?

Features	Our defined standards	Comment
Invalid RPI	**< 10 % proliferation**	**Apoptosis markers?**
Unefficient RPI	**> 20 % proliferation**	**rechallenge**
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Bacteriology control	**negative**	**Management of a positive result*?**

**Larrea L. et al., Haematologica 2004;89:1232-1237*

CONCLUSION

- In our center, most of the quality requirements were met following an almost 2 years period
- For future, our quality policy should be unchanged but…
- Should we go on with costly quality controls?

ACKNOWLEDGEMENTS

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  - Dr Poullin
  - clinic staff
- Cell Therapy Laboratory
  - Dr Veran
  - Laboratory staff
- Immunology Laboratory

DISCLOSURE

- Terumo BCT
- No conflict of interest for that topic