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Dendritic Cell Immunotherapy for Prostate Cancer

DC Program,
Biotherapy Program
and
Clinical Trials Centre

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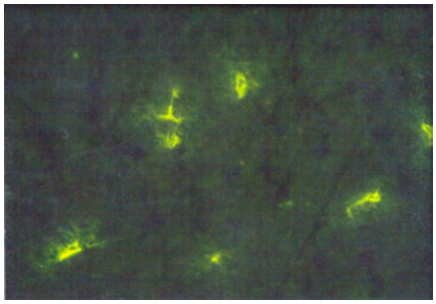
www.mmri.mater.org.au

Electricity
Iron
Flight
The Wheel
Vaccination
Fire
Computers
Cell Biology

Dendritic Cells

Dendritic Cells in Heart Tissue



J Exp Med 1981;154:347

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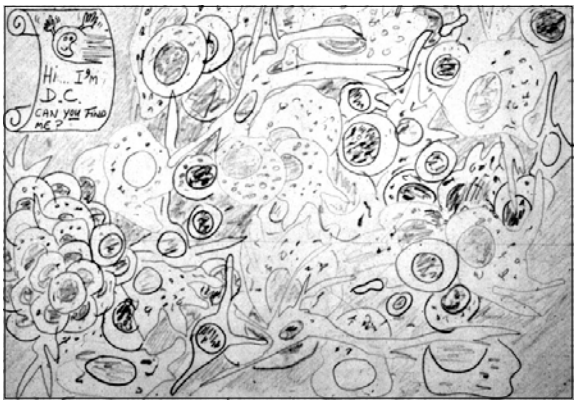
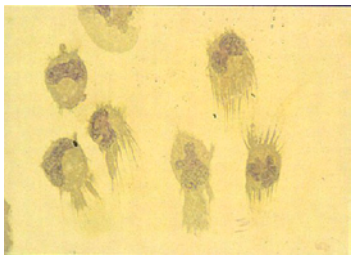
"If we understand how the dendritic cell initiates or suppresses immune responses, then we may be able to control them for therapeutic purposes i.e. increase their activity for vaccination and suppress their activity to allow tissue transplantation and control autoimmune disease."

Back at the beginning – in the late 1970s.

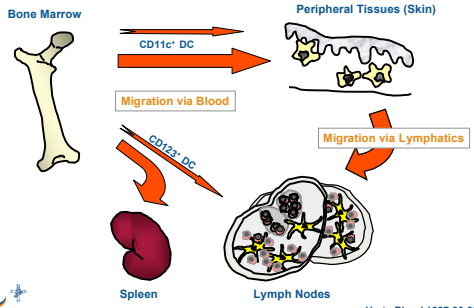


Tonsil Dendritic Cells



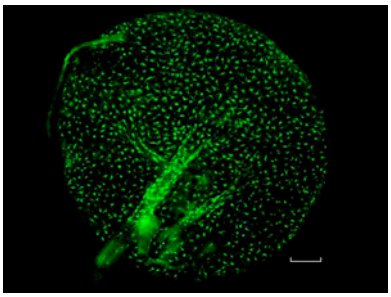
Hart & McKenzie J Exp Med 1998;165:157

Dendritic Cell Subsets and Migration



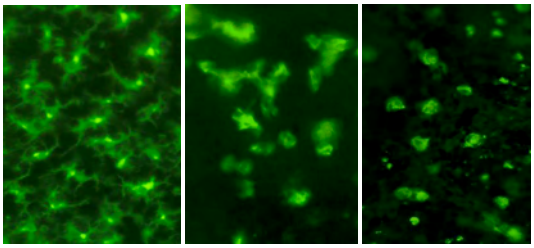
Hart., Blood 1997;90:3245

Langerhans Cells in Human Skin



Collin et al. J Exp Med 2006;203:27

Langerhans Cell Migration from Cultured Epidermis

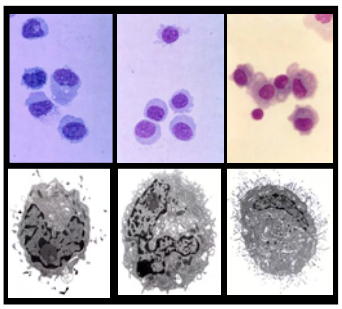


0 hrs 24hrs 48hrs

CD11c BDCA2 Mo-DC

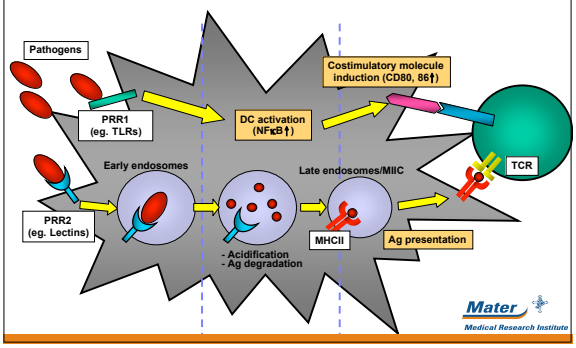
Human DC Subsets have Different:

- Precursors
- Migration
- Cytokine Profile
- Costimulation
- Survival



Hart. Pathology. 2001;33:479

Dendritic Cell Activation and Antigen Uptake



Exploiting our Knowledge of Dendritic Cell Biology

- Improve vaccination strategies - final common pathway for all is a dendritic cell.
- Correct the failure of the immune system in cancer patients?
- Control immune reactions to facilitate tissue transplantation?
- Modify auto immune reactions e.g. childhood diabetes, psoriasis, SLE?

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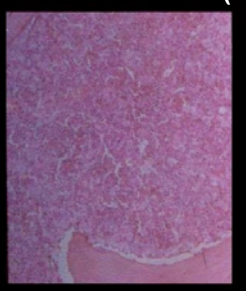
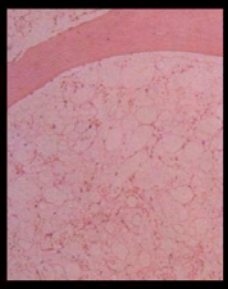
Immune Therapy for Chronic Myeloid Leukaemia (1993)



Before



After

Prostate Cancer

- Most common cancer affecting males.
- 11,200 diagnoses annually in Australia (AIHW).
- 1/11 men will be diagnosed with prostate cancer in their lifetime in Australia.
- Strong familial component.
- Treatment options are controversial (all have significant side effects).

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Treatment Options Prostate Cancer

Watchful Waiting: *The doctor may monitor the disease without active treatment.*

Radiotherapy: *Apply high frequency radiation to the gland.*

Radical Prostatectomy *Total removal of the prostate gland.*

Brachytherapy: *The implant of radio active seeding.*

Hormone Therapy: *Alterations to the hormone system, designed to reduce the testosterone level.*

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Prostate Cancer - Prognosis

Primary (curative) therapy

65% 'Cured'

35% Biochemical recurrence (PSA rise)

65% stable disease

35% Metastatic disease

(over 12% of all patients)

DC Monitoring in Clinical practice

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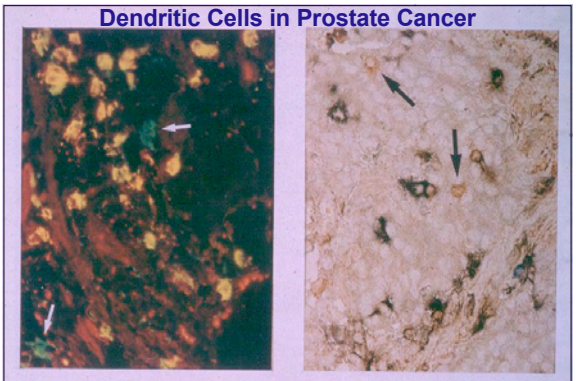
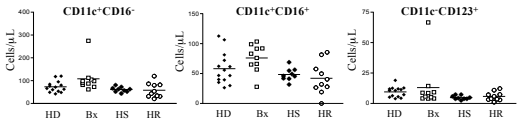
Cancers like viruses use every strategy possible to evade the immune response. Many of these affect dendritic cell function.

Dendritic Cells in Prostate Cancer



Troy et al, J Urol 1998;160:214

Dendritic Cell Counts in Prostate Cancer Patients



Wilkinson et al. Prostate 2006;66:180

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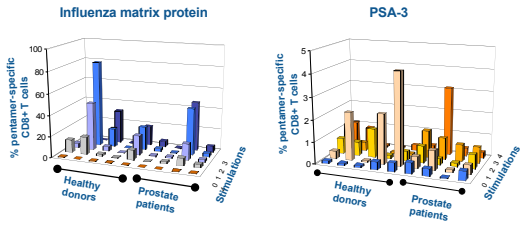
CD1c+ BDC from Prostate Cancer Patients can Induce CTL Responses



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Wilkinson et al. Prostate 2006;66:180

DC For Immunotherapy (mmri.mater.org.au)

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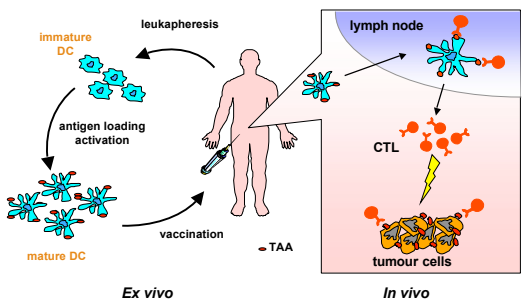
Dendritic Cell Vaccination



Apheresis to Obtain White Blood Cells

Surgeon: If whole blood comes out then whole blood goes in!

Blood Bank Director: Then how do you treat diarrhoea?

Purify DC from White Cells (0.1%)?

The Cellular Operating Theatres of the Future

Blood stem cells.

Dendritic cells.

Cytotoxic T cells.

Mesenchymal stem cells.

Skin cells.

and Gene Therapy !

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Types of DC Preparation for Immunotherapy

- **CD34 stem cell derived DC (CD34DC).**
  Require CD34+ cell mobiliization and prolonged culture in vitro.
- **Monocyte derived DC (MoDC).**
  Require extended culture in vitro and migration a concern.
- **Blood derived DC (BDC).**
  Hard to obtain in sufficient numbers and little data.

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Main Dendritic Cell Options

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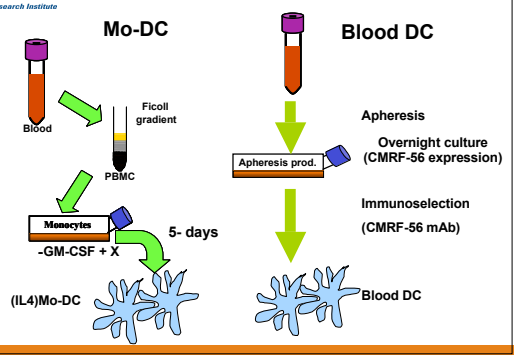

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The Patient's Blood Contains Sufficient Numbers of DC for Immunotherapy

* 15L apheresis

Lopez *et al.*, JIM. 2002;267:199

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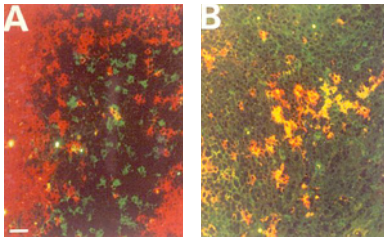
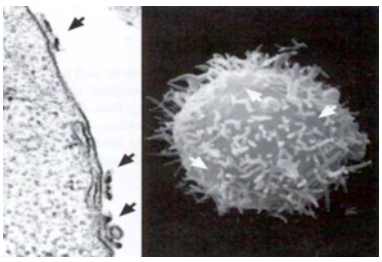
CMRF-56/CD83 Dendritic Cells in Tonsil

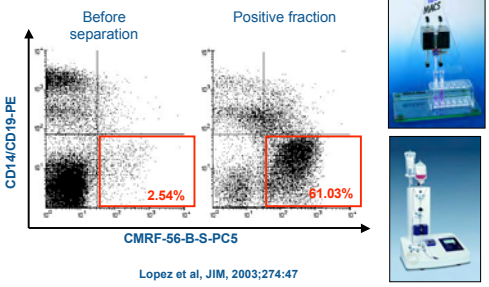


Summers *et al.* Am J Path, 2001;159:285

Dendritic Cells Labelled with Magnetic Beads

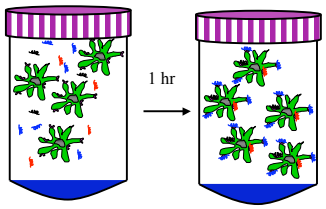


CMRF-56 Immunomagnetic Selected Enriched Blood DC (single step)



Lopez et al, JIM, 2003;274:47

Loading the BDC with Cancer Molecules



Prostate Cancer Trials

- *TWO TRIALS*
- *BDCA –1 (CD1c)(Miltenyi Biotec)*
- *Human Chimeric CMRF-56*

- Peptide antigens for DC loading
  - –PSA-3
  - –PSMA-2
  - –PAP-5
  - –Flu matrix peptide (control)
  - –Keyhole limpet Hemocyanin (control)
- Metastatic Hormone Refractory Disease
- 3 vaccinations one month apart

CliniMACS Isolation of CD1c BDC: Clinical Preparations on Normals

Healthy donc	% BDCA-1+ CD19- prio	BDCA-1 DC i starting pro	BDCA-1 Yield	BDCA-1 Purity	No. cells isolated fractior
1	0.88	4.60E+08	30%	92%	1.48E+07
2	1.51	2.20E+08	14%	93%	3.22E+07
3	1.70	2.23E+08	93%	93%	5.36E+07
4	1.17	1.99E+08	90%	90%	4.90E+07
5	0.90	3.51E+07	84%	84%	2.30E+07
6	1.00	1.18E+08	74%	74%	2.41E+07
7	0.64	2.70E+07	24%	75%	1.00E+07

Prue et al.

Prostate Cancer Phase 1 Studies

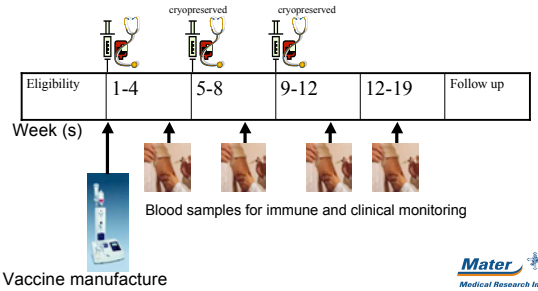
- **Primary objective:**
  - **is this novel DC preparation safe?**
- **Secondary objectives:**
  - **Does the vaccine induce an immune response?**
  - **Is there any evidence of a clinical response?**

Inclusion Criteria

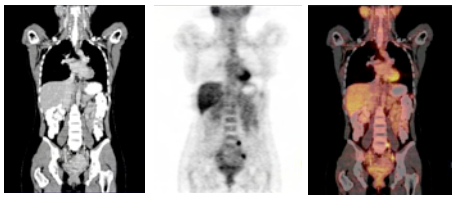
- Histologically proven adeno CA of prostate.
- Metastatic HRPC as previously defined.
- Age 18-80 years.
- Performance status ECOG ≤2.
- Adequate haematological reserves for apheresis and DC preparation.
- **HLA-A*0201 positive (40%).**
- Written informed consent.

Vaccination Schedule for DC Immunotherapy

3 vaccinations with DC/ medical monitoring (2d after vaccine)



New Clinical Imaging Techniques (CT,MRI,PET)



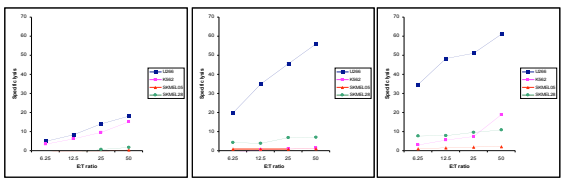
CT/PET : Recurrence of Non Hodgkins Lymphoma in the ovarian region

D9901: DC Immunotherapy for Prostate Cancer (DD9902B underway)

- Progressive androgen independent metastatic prostate cancer by bone or CT scan.
- ECOG performance status 0 or 1 with no disease related pain.
- Density gradient blood DC loaded with rGM-CSF/PAP (APC8015).
- 127 subjects randomized in 2:1 ratio to receive activated DC or control every 2 weeks for 3 doses.
- Time to progression 11.1 v 10.0 weeks (p=0.61 log rank). Gleason scores of 7 or less 16.1 v 9.1 weeks (p=0.001 log rank) with higher probability of remaining free of cancer related pain.
- Median interim survivals (Gleason 7 or less) were 30.7 months with APC 8015 versus 22.3 months with control (crossover allowed).

Schellhammer et al. World J Urol 2005;23:47

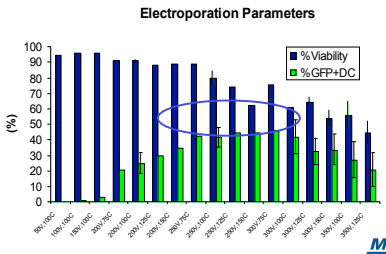
Activated BDC Induce Strong Anti Myeloma (U266) CTL Responses



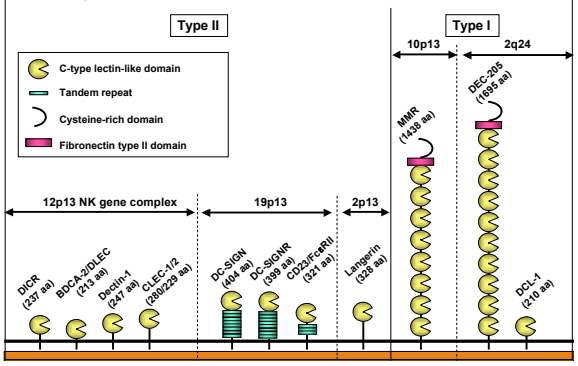
BDC | BDC + CD40L | BDC + GM-CSF

Freeman et al. In preparation.

Viable RNA Loaded CD34⁺DC Require Electroporation at Low Voltage



C-type Lectin Receptors on DC (partial list)



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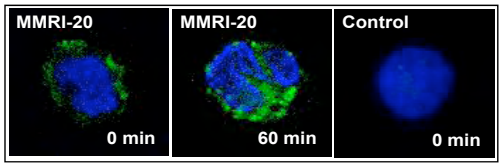
Human Myeloid BDC Internalize CD 302 (DCL1)

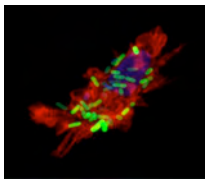


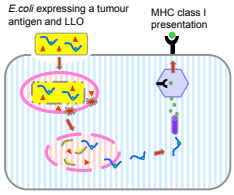
Freshly isolated BDCA1 selected BDC were incubated for 60 min with FITC mAb.
The nuclei are stained with DAPI.

Kato et al. In preparation.

Recombinant Bacteria as a Delivery Vehicle for Tumour Antigens to DC

DC loaded with recombinant *E.coli* expressing LLO and MelanA for immunotherapy?



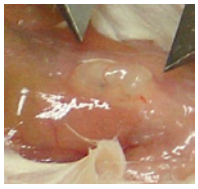


Radford et al

Animal Models to Refine Therapeutic Options

Too many opportunities for phase 1 (phase 2 and phase 3) clinical trials.

Too many combinations to test.



Cooperative trial programs

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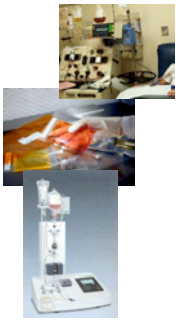
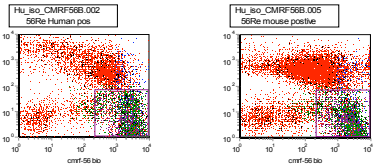
CMRF-56 BDC Immunotherapy

White cell collection (leukapheresis)
Overnight culture of unficolled cells
biotinylated CMRF-56 Antibody staining
Anti Biotin CliniMACS beads
Selection on CliniMACS device
Load DC with tumour antigen
Vaccinate patient | Cryopreserve



Human Chimeric IgG4 CMRF-56 mAb Purifies BDC



Humanised chimeric CMRF-56 antibody:
DC purity: 39%

Mouse CMRF-56 antibody:
DC purity: 32%

Vari et al. In preparation.

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Production of CMRF-56 Antibody

Predictions:

DC targeting *in vivo.*

Diagnostic and therapeutic tumor lab report.

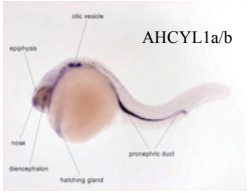
Flexible TAA off the shelf delivery system.

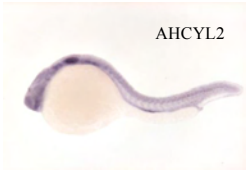
Hospital based.

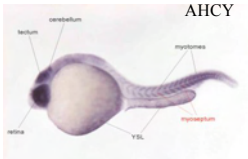
Integrated with chemotherapy treatment.

$$$$$$$$$

DC Targeted Therapeutics





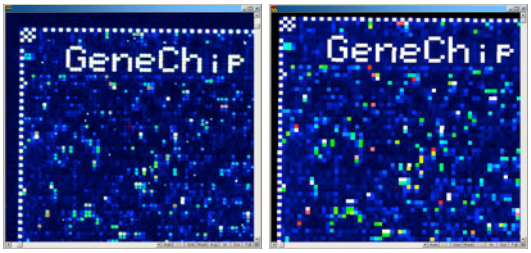


Quantitative RT-PCR shows AHCYL1 expression is spatially restricted but AHCY and AHCYL2 are not (Thisse et al).

Gene Chips in the Diagnostic Process

Pre image smoothing (Raw Data)

Post image smoothing (Quantifiable data)



Conclusions

We have much to learn about DC biology.

Reagents to detect DC and define their functional state are increasing.

Methods to count and monitor DC are now available.

DC are a practical option for immunotherapy and may be targeted *in vivo.* Combined immune manipulations may be effective. Immunotherapy is compatible with chemotherapy?

Targeting DC for immunosuppression (another story).

Acknowledgements

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Gambro

Miltenyi

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Philanthropic Support


Gates steps aside

Nick Papps
IN LOS ANGELES

BILL Gates, the world's richest man, is giving up his day job to give away billions of dollars.

Mr Gates, the founder of software leader Microsoft, will quit the day-to-day running of the software giant to work full time on his charitable foundation.

Your potential.

RESPONSIBLE ... Bill Gates feels a responsibility of wealth to give back to society.

Mater Foundation

New Medicine:

academically driven medical practice involving large teams lead by scientists competing fiercely to get the best outcomes for the limited dollars provided by socially responsible funding.

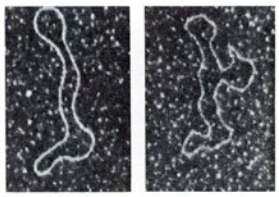
Key Advances in Molecular Biology

Bugs to replicate and clone individual DNA fragments.

Enzymes to cut and glue DNA.

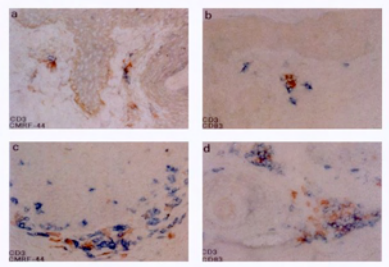
DNA amplification systems.

DNA sequencing.



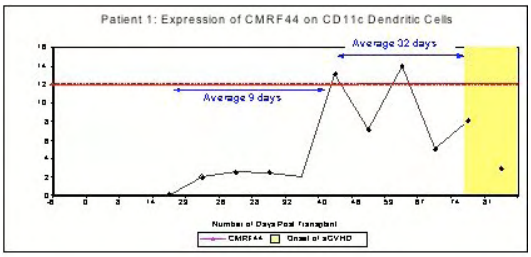
End of Lesson

Activated CMRF-44 DC – T Cell Interaction in the Dermis



McLellan et al., J Invest Dermatol. 1998;111:841

CMRF44 Expression Prior to AGVHD



Lau et al, submitted.

Clinical Trials

Over 126 reports to date. (www.mmri.mater.org.au).

Some tentative conclusions:

- Immature Mo-DC prepared in GMP conditions are not be suitable.
- Intravenous administration may be less effective.
- Cell dose studies are inconclusive.
- Ongoing vaccination (schedule unknown) is needed.
- Cytotoxic responses correlate with clinical response.
- Few Mo-DC migrate to draining nodes.

Other conclusions:

- Most studies use Mo-DC (GM-CSF plus IL- 4 (IL- 13, IFN)).
- Maturing agent and time for maturation?
- Tumor lysate/ peptides/ other TAA.

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Combination Active DC Immunotherapy and anti VEGF Therapy in Prostate Cancer

- Prostate cancer patients, who had undergone definitive therapy prior to disease progression. No hormonal therapy apart form in the adjuvant setting
- APC 8015 – APC (DC) incubated with PAP-GM-CSF.
- 3 IV DC infusions given 2 weekly with Bevacizumab (anti VEGF, Avastin),10mg/kg IV following. Further Bevacimab 2 weekly thereafter.
- 22 patients enrolled, 21 evaluable. 9/9 increased T proliferation.
- Reductions in PSA in 9 (3 >25%). Median PSA doubling time extended from 6.9 months to 12.7 months.
- 6 Grade 3 toxicities – therapy discontinued in 4.

Rini B I et al. Cancer. 2006;107:67

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A Randomized Phase 3 Trial of DC Immunotherapy in Metastatic Melanoma

- Stage IV melanoma patients.
- Monocyte derived DC matured with cytokine cocktail.
- Loaded with multiple class 1 and class II helper peptides.
- DC given s.c. 2 weekly (x5) then every 4 weeks.
- 108 subjects randomized between DC (53) or dacarbazine (DTIC).
- DC vaccination could not be demonstrated to be more effective than DTIC.
- HLA-A2+/HLA-B44- haplotype survived significantly longer than other HLA haplotypes.

Schadendorf D et al. Ann Oncol. 2006;17:563

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Clinical Grade CMRF-56 mAb BDC Preparations

	CMRF-56 concentration		Acceptable Range for phase I Trial
	50ug/ml	25ug/ml	
% DC before	1.1	1.4	NA
Yield DC	41	40	20-100
Purity DC	21	35	10-100
% Viability	74	86	60-100

Table 2: The yield, purity and viability of CMRF-56+ cell populations using the clinical grade CMRF-56 antibody.

Vari et al. In preparation.

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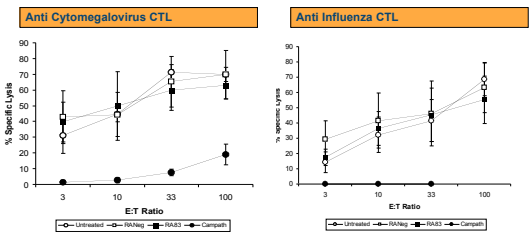
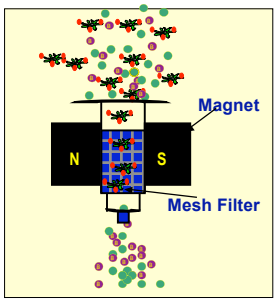
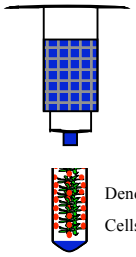
Anti Viral CTL Responses after Anti CD83



Are preserved in contrast to after Campath1 treatment

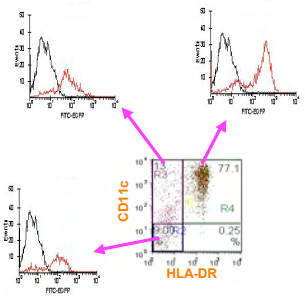
Magnetic Isolation of Blood Dendritic Cells



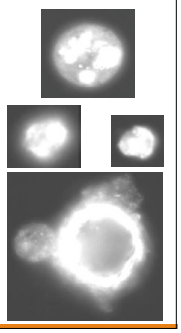


Dendritic Cells

CD34 DC Loading with GFP mRNA



… and total Leukaemic RNA



DC and Tumour Immunity

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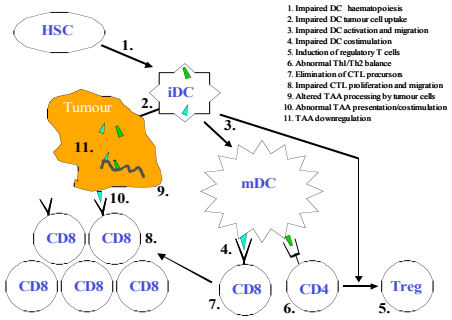
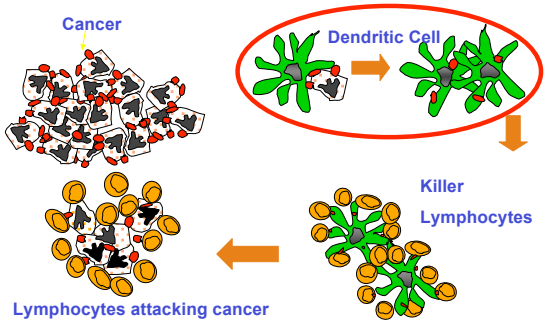
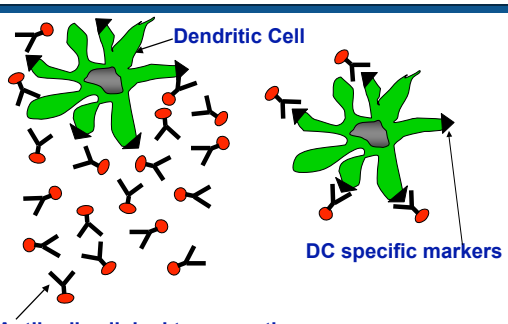


1. Impaired DC haematopoiesis
2. Impaired DC tumour cell uptake
3. Impaired DC activation and migration
4. Impaired DC costimulation
5. Induction of regulatory T cells
6. Abnormal Th1/Th2 balance
7. Elimination of CTL precursors
8. Impaired CTL proliferation and migration
9. Altered TAA processing by tumour cells
10. Abnormal TAA presentation/costimulation
11. TAA downregulation

DC and Cancer





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