IN VIVO TRACKING DENDRITIC CELLS IN PATIENTS WITH MULTIPLE MYELOMA

Miles Prince[1*], Dominic M Wall[1*], David Ritchie[1*],Dirk Honemann[1*], Simon Harrison[1*], Hang Quach [1*], Mick Thompson[2*], Rodney Hick[2*],Eddie Lau[2*], Jill Davison[1],Maureen Loudovaris[1*], Jude Moloney[1*], Bruce Loveland[3], Jacques Bartholeyns[4*], Andrew Katsifis[5*], Linda Mileshkin[*]

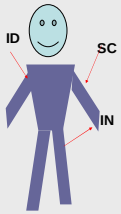
[1] Peter MacCallum Cancer Centre, East Melbourne, Australia, 3002
[2] PET Centre, Peter MacCallum Cancer Centre, East Melbourne, Australia, 3002
[3]The Austin Research Institute, Heidelberg, Australia, 3084
[4] IDM, 172 rue de Charonne, 75011 Paris, France
5 ANSTO Radiopharmaceuticals and Industrials, Sydney, Australia.

Aim

The objective of this study was to determine the in-vivo distribution of dendritic cells (DC) when given to patients (pts) with Multiple Myeloma (MM) via varying routes of injection (intravenous- IV, intranodal- IN, intradermal-ID and subcutaneous-SC) by using DC labelled ex vivo with ^{111}Indium (In)-Oxine (n=6 pts), ^{18} Fluorine fluorodeoxyglugose (FDG), or ^{64}Copper pyruvaldehyde bis-N-4-methylthiosemincarbazone [^{64}Cu-PTSM (n= 2 pts)].☐Eligible pts had stable or slowly progressive MM not requiring systemic therapy with evidence of increased MUC-1 expression. Autologous monocytes were collected from each patient via apheresis and dendritic cells were produced by in vitro culture within the VacCell (IDM SA) processor.

Method

Following apheresis, monocytes were cultured for 7 days with GM-CSF and IL-13. On days 0 and 4 they were pulsed with MUC1-Mannan fusion protein (24h) prior to use. DC were then cryopreserved non-matured (imDC) or matured (mDC) with 1 g/ml FMKp and 500 UI/ml gamma IFN (6h) and then frozen. Pts received DC initially IV and then two weeks later by each of IN, SC, ID simultaneously. Pts at each DC administration were serially imaged using planar gamma camera for up to 4 days.



Prior to injection, DC were radio-labeled with either ^{18}FDG, ^{111}In-Oxine, or ^{64}Cu-PTSM. These were then resuspended to administration volume with 5% serum/saline solution. IV administration volume was 100mL, IN & SC was 1.0mL and ID was 0.5mL.

ID & SC injections were administered in the lateral shoulder region on contralateral sides. IN injections were injected into an inguinal lymph node under ultrasound guidance. Two weeks later,cells were administered by IV infusion (100ml)

Ex Vivo Radiolabelling

DC were incubated at room temperature in a 5% autologous serum/saline solution for 45-60 minutes after addition of ^{111}Indium oxine and washed twice before resuspension ready for administration. Cells were labelled with In111 (20 MBq IV or 0.7 MBq maximum each of IN, ID and SC)

Median Cell Numbers

IV Administration	**$1.5x10^8$ (range = 0.33-$2.5x10^8$, n=6)**
IN/SC/ID Injection	**$2.0x10^7$ (range = 1.3-$3.6x10^7$, n=6)**

Summary of patient cohorts

Patients were entered in 3 cohorts. Cohort 1 consisted of 3 patients who received injections with nmDC, cohort 2 has 3 patients whor received mDC. Cohort 3 was later added in whom 2 patients received ^{64}Cu-PTSM

Cohort	Patient ID	Age/ Sex	Durie-Salmon Stage	Number of prior regimens	Prior high-dose therapy	Type of DC
1	Pt001	47F	3A	3	AuSCT	nmDC
	Pt002	61M	2A	2	N	
	Pt003	68F	2A	4	AuSCT	
2	Pt004	51M	2A	7	AlloSCT	mDC
	Pt005	67F	3A	2	AuSCT	
	Pt006	55M	2A	2	AuSCT	
3	Pt003[R] Pt003[R2]	69F	3A	4	AuSCT	mDC
	Pt006[R]	55M	2A	3	AuSCT	

F, female; M, male; AuSCT, autologous stem cell transplant; AlloSCT, allogeneic stem cell transplant; N, no; R, re-enrolled into later cohort

ID, SC Administration

^{111}In-Oxine

Consecutive planar images in one patient injected with mDC labelled with ^{111}In-oxine after simultaneous ID and SC administration with evidence of axillary tracking by day 1. A) left anterior oblique statics of ^{111}In-labelled mDC, following ID injection into left deltoid region, axillary node demonstrated. B) Right anterior oblique statics ^{111}In-labelled mDC, following SC injections into right deltoid region, axillary node demonstrated.

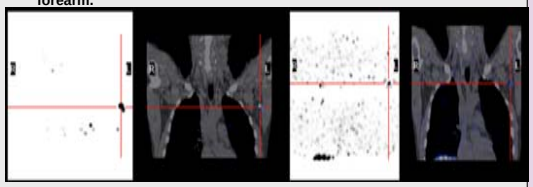
Cell Preparation	4 hours	24 hours	48 hours	72 hours
mDC SC	0.06%	0.22%	0.74%	1.30%
mDC SC	0.10%	0.40%	0.67%	0.73%
imDC ID	0.01%	0.14%	0.30%	0.48%
mDC ID	0.02%	0.26%	0.43%	1.08%
mDC ID	0.36%	0.80%	0.83%	1.29%

nmDC migrated to LN in 1/3 ID & 1/3 SC injections whilst mDC tracked to LN in 2/3 ID injections & 3/3 SC injections.

Quantitative analysis of tracking was possible in 5/7 of the serial images (table left).

Minimum number of cells definitely demonstrable within a node was shown to be 22 x 10^3 cells

^{64}Cu-PTSM: ID administration resulted in cell migration in 2 patients. Below is a coronal PET/CT images showing presence of activity within axillary lymph node at 2 hours (left) and 20 hours (right) after ID injection into volar aspect of left forearm.



Results of regional nodal tracking acording to route of administration

Patient ID	DC Type	Labeling method	Intradermal	Subcutaneous	Intranodal
Pt001	nmDC	^{111}In-oxine	Y*	N	N
Pt002			N	Y	N
Pt003			Y	N	N
			2/3	1/3	0/3
Pt004	mDC	^{111}In-oxine	N	Y	N
Pt005			Y*	Y*	N
Pt006			Y*	Y*	Y
			2/3	3/3	1/3
Pt003[R2]	mDC	^{64}Cu-PTSM	Y*	Y	N
Pt006[R]			Y*	N	N
			2/2	1/2	0/2

Shaded area is matureDC.
*Quantification studies performed (see Table 6)
nmDC, non-matured dendritic cell; mDC, mature dendritic cell; Y, Yes; N, No.

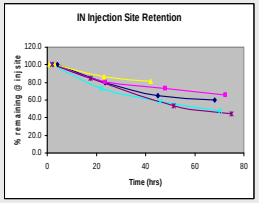
IN administration

^{111}In-Oxine

IN injections of ^{111}In-Oxine labeled DC resulted in immediate LN disruption with labled cells being evident in adjacent nodes within 4 hours of injection. The rapid migration was considered to be evidence of disruption of node at time of injection drather than definitive cell migration.

In 1/3 Non-mDC and 2/3 mDC there was early and substantial drainage into an afferent LN (<4 hours, >3% of cells).

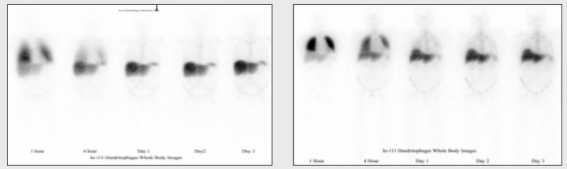
Activity cleared from the administration site at a median rate of ~20% per day, probably due to radiolabel efflux rather than cell migration (table left)



^{64}Cu-PTSM: In contrast to ^{111}In-Oxine, nodisruption to lymph node capsules was seem. However, no evidence of proximal lymph node migration was observed.

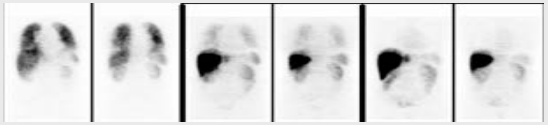
IV Administration

^{111}In-Oxine: No Tracking to peripheral lymph nodes was demonstrated with either mDC or nmDC following IV injections



Consecutive whole body planar images after IV administration of ^{111}In-oxine labeled DC acquired at 1h, 4h, 1 day, 2 days and 3 days with evidence of
Initial migration of cells to lungs followed by rapid redistribution to liver and spleen with nmDC (left panel), and slower redistribution to liver, spleen and bone marrow with mDC (right panel).

^{64}Cu-PTSM: No tracking to peripheral lymph nodes was demonstrated in 2 mDC IV administations.



Consecutive coronal PET slices from whole body acquisition of ^{64}Cu-PTSM labelled mDC following IV administration at 1 hour (2x left images), 3 hours (2x middle images) and 18 hours (2x right images)

Summary of infusion episodes, tracking studies and immunological outcomes

Conclusion

^{111}In-Oxine and ^{64}Cu-PTSM gives reproducible tracking of both nmDC and mDC to regional LN following either ID or SC administration, with mDC revealing superior migration to regional LN.