

MONICA

Exhibit 8
Market Intelligence on Baxter Isolex 300SA and Isolex 300i

Seminar at Peter MacCallum Hospital, Melbourne, Australia.

Report by Boon Yap - 26 Nov 1996

Summary

In the past four months, Baxter has been more active in Australia. They held a technical workshop for the Association of Bone Marrow Scientists Meeting held in Adelaide and placed Isolex 300i instruments at the Royal Adelaide Hospital and Royal Prince Alfred Hospital.

I attended their seminar held at Peter MacCallum Hospital on invitation by the hospital's staff.

Baxter Biotech Division held a 2 hour seminar on three topics:

1. Stem collections on the CS3000 - Stacie Simpson, Product Support for CS3000.
2. Mobilisation Sequential Evaluation of Cytokine in Normal Donors - Dr. Louise Konkuli, Assistant Clinical Director, Baxter Immunotherapy.
3. Technical Aspects and Clinical Experience with Isolex 300 (SA and i)- Dr. John Anodyne, Director of Scientific Affairs.

The Isolex 300i is no faster than the CEPRATE[®] SC System. It gives very similar yields, with higher purity reported. However, the data is controlled with high starting CD34%. Similar trends reported for low starting CD34%. Their final product volume is 100 ml.

They did not present anything new with their clinical data. Generally studies were ill defined. There is insufficient numbers to really back up some of the claims for reasons reported for delayed platelet engraftment. Data presented were at times sketchy and unclear. They often mixed data obtained using the SA with the i instrument.

There is no randomised trial to date. The trial reported for the b cell malignancies were a mix of diseases. There was no tumour purging data shown for BC study.

There was no clinical data to show safety of reinfused beads except that mentioned for animal toxicity studies.

However, the automated wash procedure does present well against our manual equivalent. They are price competitive with AUD \$20,000 for the Isolex 300i instrument and an approx. total of AUD \$3000 for the disposables.

NB: The following report is retold as accurately as presented Only topic number 3 is reported here.

3a. Technical Aspects with Isolex 300 (SA and i)

Baxter first focused on negative depletion but in the last 4 to 5 years they concentrated on CD34+ selection. They must see virtue in the latter.

They described the Isolex 300i at length, referring to the Isolex 300SA as obsolete. In the rest of the report unless otherwise stated, notes are on the 300i.

They still use a double antibody set-up. The primary antibody 9C5 is a class II murine anti-CD34 antibody. The secondary antibody with the magnetic Dynal bead attached is a sheep anti-mouse antibody.

A Four Step Process

They refer to a four step process.

1. MNC - removal of platelets
2. Sensitisation with Ab
3. Rosetting
4. Isolation of cells from Ab

Baxter showed their SA model briefly, mainly to state that they have improved significantly on the problems associated with chymopapain release and the six hours processing time.

Components of the Isolex 300i:

1. Clamps - the system never use the same tubing for positive and negative selected samples.
2. Pressure monitors
3. Fluid sensors
4. Weigh scales
5. Spinning membrane for platelet wash, Ab wash and peptide wash.
6. Pumps - one each for cell source, fluid, buffer, washing.

Total processing time takes 2 hours 45 min, plus whatever time required to set-up.
They quoted a set-up time of 15 min which included the load and prime steps.

Step by step breakdown description

Step 1: Load bags to weigh scale at the top of the instrument.

Once bags are hanging, operator is not allowed to touch the bags as the computer will show weight error due to the fluctuations. Bags comprise of a separate bag for sample, peptide, Ab1, wash solution, final recirculating buffer, Ab2 and waste. They have in-line 0.2 micron on the peptide and Ab bags.

But still unclear are:

? The third bag that has an in-line filter.

What is the difference between "fluid, wash and buffer" solutions used.

Step 2: Wash cell source to remove platelets.

The spinning membrane retains the cells but platelets spin through and is removed.

Step 3: Incubation with Abl

The cell sample is kept recirculating through the lines and the spinning membrane to keep cells mixing. Abl is added during recirculation which takes 20 min. and further incubated for 15 min.

Step 4: Cell Wash Abl

Cells are washed with 300 ml washing solution to remove excess Abl.

Step 5: Rosetting

Cell solution is transferred to the chamber and rocked to optimise Ab2 binding. Rosettes are formed in the 30 minute incubation.

Step 6: Capturing of rosettes

Magnet is switched on to hold onto rosettes, and the effluent flows to waste.

Step 7: Buffer addition and wash

Three wash steps are required to reduce non-specific trapping of other cells to bound rosettes.

Step 8: Incubation PR34+

A 30 min incubation.

Step 9: CD34 recovery

The chamber port is inverted to ensure CD34+ cells are not trapped in between the rosettes. Buffer is added to chamber to collect cells.

Step 10: PR34+ wash

The recovered cells are washed 3 times to remove residual PR34+. Final product volume is 100 ml.

Data from 18 runs in the lab.

Capture: 82%

Yield: 54%

Purity: 86%

TNC of sample: 0.274×10^{10} - 5.33×10^{10}

3b. Clinical Experience with Isolex 300 (SA and i)

Baxter started clinical work on Isolex 300SA in Nov '94, initially with chymopapain. In June '95, they started using the PR34+ and found similarities in yield, purity and engraftment. As a result they (are) submitted (ing) to FDA as pooled data.

Their goals:

- To provide reliable stem cells
- Reduce unwanted cell populations
- Reduce T cells
- Without compromising neutrophil and platelet engraftment.
- To demonstrate no adverse reactions associated with reinfusions.

Initial studies were on BM.

Clinical studies

1. Autologous BC - Isolex 300SA, Isolex 300i?
2. Autologous B cell malignancies- Isolex 300SA, Isolex 300i?
3. Allogeneic BM - Isolex 300SA, Isolex 300i
4. Allogeneic PBSC

1. Autologous BC study

Pivotal PhII study now, multicentre, \pm CD34+ selection, advance stage IV metastatic or high risk stage II and III \geq 4 nodes.

Primary endpoint: safety

Secondary endpoint: infusional toxicity (competitor showed reduced toxicity for BM, they wanted to show is same for PBSC) and efficacy for tumour removal.

Criteria for study

1. 20 CD34 cells/ul
2. variety of mobilisation regimes (5/6 sites with G-CSF)
3. not randomised (sequential?)
4. all received G-CSF post-transplant

n = 216

patient no. = 108

average starting CD34% = 1.2%

Yield = 40%

Purity = 90%

Chicago

Starting material

	CD34 cells/kg	Backup	# Products
Selected	5×10^6	1.5×10^6	3 (1-5)
Non selected	2.5×10^6	none	2 (1-6)

n=64, with 26 ineligible due to insufficient cells mobilised, most of which had high number of pre-treatment.

Engraftment

	CD34 cells/kg	ANC > 500 ul	Plt > 20,000 ul
Selected, n=20	2.3 (0.8-16)	11 (9 - 21)	12 (8 - 41)
Non selected, n=20	4.7 (2.7 - 38.3)	similar	similar

Conclusion: No significant difference between selected and unselected arm wrt to ANC and platelet. 90% patients recovered by day 15.

Infusional toxicity

	volume product	volume DMSO	
Selected, n=20	100 ml	10 ml	Associated with clinically detectable side effects.
Non selected, n=20	385 ml	39 ml	Not associated with infusional toxicity. Do see nausea and tachycardia

They also looked at time to relapse, seeing some increase in time to relapse for the selected arm, but admitted trial was not designed nor significant at the 206 days time point.

2. Autologous B cell malignancies

n= 39. Not randomised (compared with historical?). The objective is to reconstitute CD34+ cells. Patients with very heterogeneous diseases. They achieved a 2 - 3 log removal of B and T cells; with 20% contamination reduced to 2% and 1% reduced to 0.04%.

Average CD34 dose = 3.6×10^6 /kg

Engraftment recoveries for ANC 6 - 26 days: for platelet 6 - 30 days (those that took longer were treated with Bactran).

No major risk of adverse reaction.

3. and 4. Allogeneic BM and PBSC

(Things got a bit confusing here where same data was presented twice. It was not possible to distinguish Isolex 300SA from 300i data, nor the BM from PBSC data)

Bill Bensinger on acute leukaemia.

Criteria for study

1. Acute leukaemia that had relapsed, accelerated blast phase CML, 1/3 patients were lymphoma and myeloma.
2. Some were mismatched
3. Mean age = 42 years
4. G-CSF given post transplant
5. MTX only to mismatched

Isolex 300SA

n = 69 (yields and purity shown were for Isolex 300i)

Follow up to date is 500 days. One died by 100 days due to relapse CML, five mismatched died due to infections. One mismatched patient was dead by 50 days due to primary infection, aspergillus. Patients who died were usually heavily pre-treated.

Long term follow-up is up to a year, GVHD:

n = 23, acute GVHD

n = 13, chronic GVHD

n = 33, do not have symptoms yet

GVHD status	acute	chronic
NR	2	6
0	7	2
1	6	2
2	6	3
3	2	0
4	0	0

Isolex 300i

In the past 6 months they have enrolled 32 patients: n = 29, 14 on old software, 9 on new software.

Average CD34 dose = 6.2×10^6 /kg (range 2.6 - 10.1)

CD3+ remain = 0.15×10^6 cells/kg (range 0.04 to 0.55)

Purity 87%, yield 47%.

T cell removal as measured by CD3+ cells = 3.2 log, achieving 2×10^5 to 6×10^5 T cells/kg.

Engraftment recoveries for ANC 10 - 15 days, for platelets average 20 days. Some platelet recoveries were earlier than ANC. They think this was due to not getting G-CSF post transplant (in practice half the patients were not treated) thought all were post-treated), whilst delayed was thought to be due to methatrexate treatment. In the absence of methatrexate they see equivalent engraftment.

0-250 day follow-up. Only in some groups could they evaluate GVHD
 Concluded can demonstrate the use of Isolex for mismatched setting (!?).

GVHD status	acute	chronic
NR	4	3
0	3	6
1	1	1
2	7	2
3	1	0
4	1	0

Next study

randomised, \pm CD34 selection with different GVHD prophylaxis.

Audience question and answers

- Post processing samples:
 CFU-GM data 80 - 85%
 LTC-IC not yet set up test in-house
- CD34 dose: $< 2.5 \times 10^6$ CD34+ cells/kg not recommended due to variability in flow assay and delayed engraftment, saying with BM, can engraft with half the amount.
- Subset Analysis: They have only done in-house subset analysis on normal donors. They are now collecting data from sites, say they do not see a difference.
- Getting enough CD34 numbers?:
 Recommend pooling 2 collections.
 Recommend storing with autologous plasma.
 Not recommending processing of frozen sample - clumping. Some investigators are looking into this.
- Not recommending storage for >24 hours, viability and clumping problems.
- Capacity: When validated in-house can process up to 5×10^{10} TNC; they think can perform 8×10^{10} but have not done this
- Beads required: The process needs 4×10^9 beads, a ratio of 10 beads: 1 CD34 cell, regardless of procedure numbers.
- Starting sample limitation: $<0.5\%$ (20 CD34/ul in PBSC) starting CD34, results are much more variable where yields tend to be lower.
- Mobilisation: They encourage the use of chemo and G-CSF. B cell myeloma mobilise well. BC data was from different mobilisation regimes, 20% would not mobilise and is usually associated with more prior treatment.

Key Discussion Points

1. They admitted beads do escape from the second magnet of the Isolex 300SA ending up in final product.
2. Isolex 300i releases more beads than Isolex 300SA. Figure quoted is 2000 beads per procedure for Isolex 300L, but less for the SA.
3. Their toxicity studies with mice and rats do not show any significant problems, beads are removed by the reticuloendothelial system, the LC50 is \approx 2,000,000 beads.
4. They claim no HAMA or HASA.
5. Significantly brighter cells on flow-cytometry when compared to the chymopapain released product, no change in fluorescence suggesting no change in CD34 cell surface antigen.
6. If operator want to do negative selection, they can collect the product in a separate bag.

Own questions:

Not sure how the instrument could combine positive selection and negative depletion.