Sickle Cell Disease: A Look to the Future

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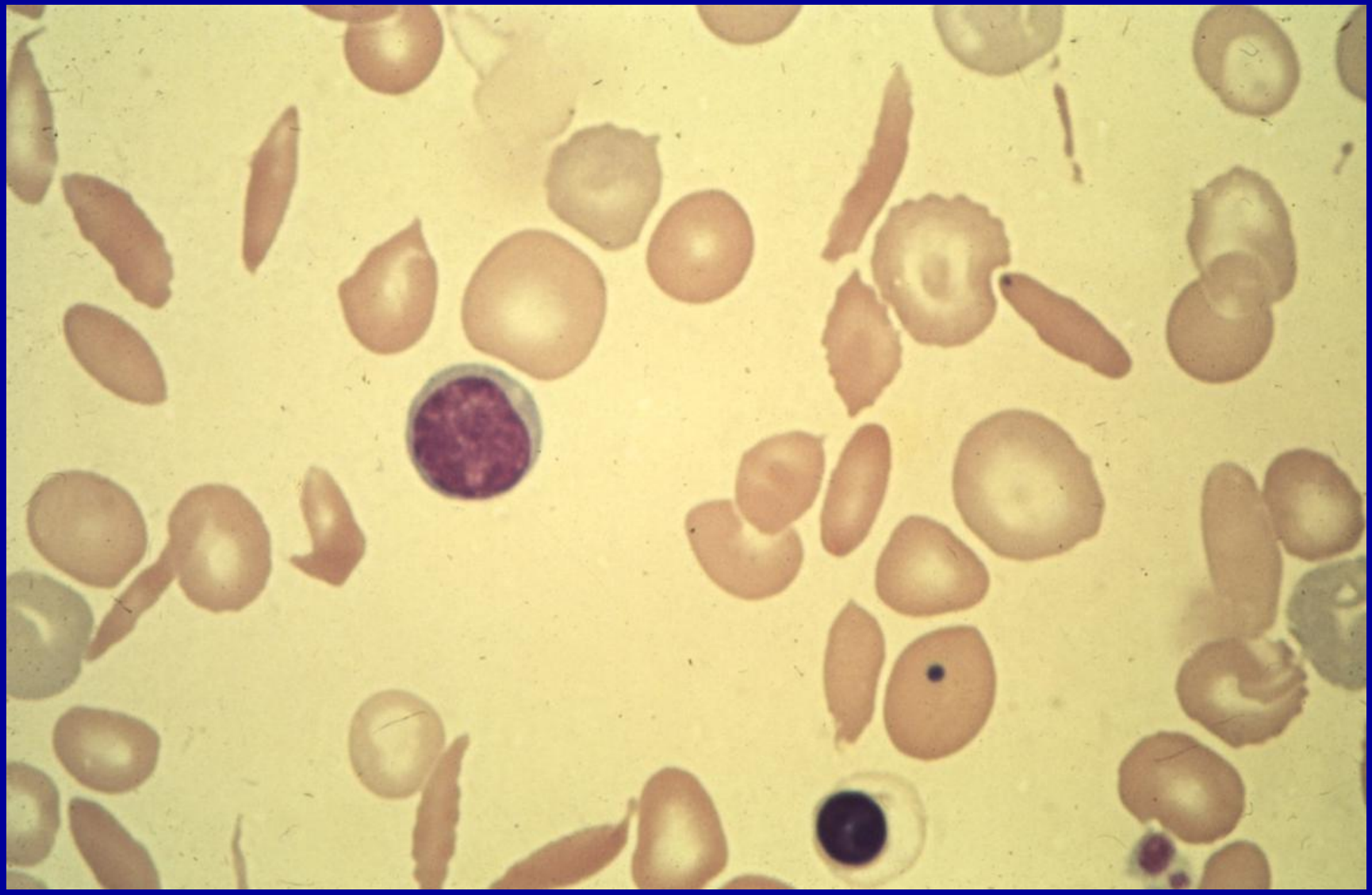
Industrial Relationships

- Scientific Advisory Board: Aileron, Inc
- Consultant: NK Therapeutics, Inc


NORMAL
SICKLE



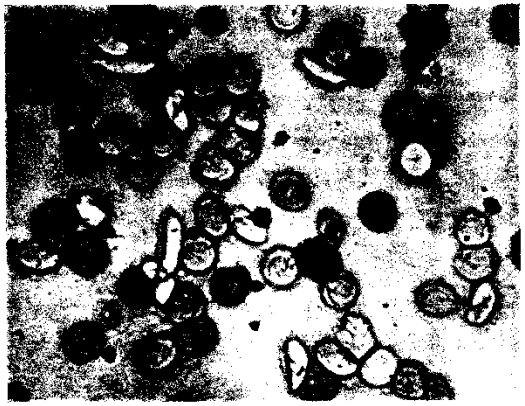


FIGURE 2 Photomicrograph of Hb SS erythrocytes stained for Hb F by the Kleihauer-Betke technique.

ture of amino acids-^{3}H, approximately 200 μc/100 ml of blood, (reconstituted protein hydrolysate, tritiated, Schwartz Bio-Research, Orangeburg, N. Y.) was used as the isotopic precursor of hemoglobin. Cover slip preparations from oxygenated samples of venous blood removed at approximately daily intervals were fixed in absolute methanol for 10 min, mounted faceup on glass slides, dipped in Kodak NTB-2 nuclear track emulsion, and exposed for 3–5 wk. The developed, fixed, and stained preparations were then examined for grain-bearing erythrocytes.

Method 3. Incubation, reinfusion, and sampling of blood from Hb SS patients were performed as in Method 1 except that $Na_2{}^{51}CrO_4$, approximately 50 μc/100 ml of blood, was used as an isotope.

RESULTS

Clinical data and observations. Routine hematological data on the patients used in these studies are shown in Table I. In no patient did present or past symptomatology correlate with total Hb level or per cent Hb F. Scattergrams were constructed of Hb level vs. per cent Hb F, of Hb level vs. per cent ISC, and of per cent Hb F vs. per cent ISC: no correlations were found in the first two, but per cent Hb F correlated inversely with per cent ISC ($r = 0.65$).

Photometry of single cells. Optical densities (50 sickled and 50 nonsickled cells per slide, selected at random) were measured on cover-glass preparations stained by the Kleihauer-Betke tech-

TABLE II

Optical Densities of Hb SS Erythrocytes Stained for Hb F

Patient	Irreversibly sickled cells	Nonirreversibly sickled cells	P
1	0.034 (±0.019)*	0.068 (±0.047)	<0.01
2	0.033 (±0.013)	0.071 (±0.036)	<0.01
3	0.034 (±0.018)	0.091 (±0.071)	<0.01
4	0.026 (±0.019)	0.058 (±0.035)	<0.01
Mean	0.032 (±0.004)	0.072 (±0.014)	<0.01

* Mean (±SD) of 50 cells.

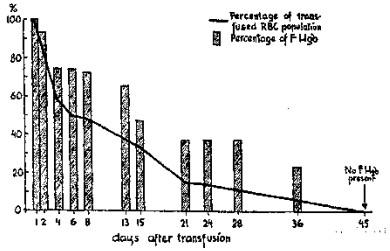


FIG. 2. Disappearance of transfused sickle cell anemia cells containing 16.8 per cent F hemoglobin. Recipient 2. Initial post-transfusion values (100 per cent): cells, 671,000/cu.mm.; F hemoglobin, 4.4 per cent.

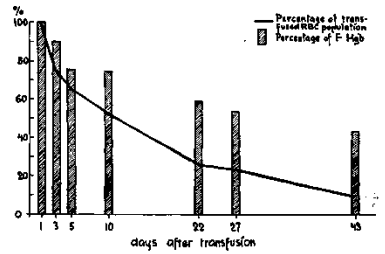


FIG. 3.—Disappearance of transfused sickle cell anemia cells containing 16.5 per cent F hemoglobin. Initial post-transfusion values (100 per cent): cells, 830,000 cu.mm.; F hemoglobin, 4.9 per cent.

denaturation value of the recipient was always deducted from the amount of F hemoglobin found. Follow-up studies were carried out as frequently as feasible, particularly during the first two weeks after the transfusion (figs. 1 to 4). The children were only hospitalized for the performance of the transfusion.

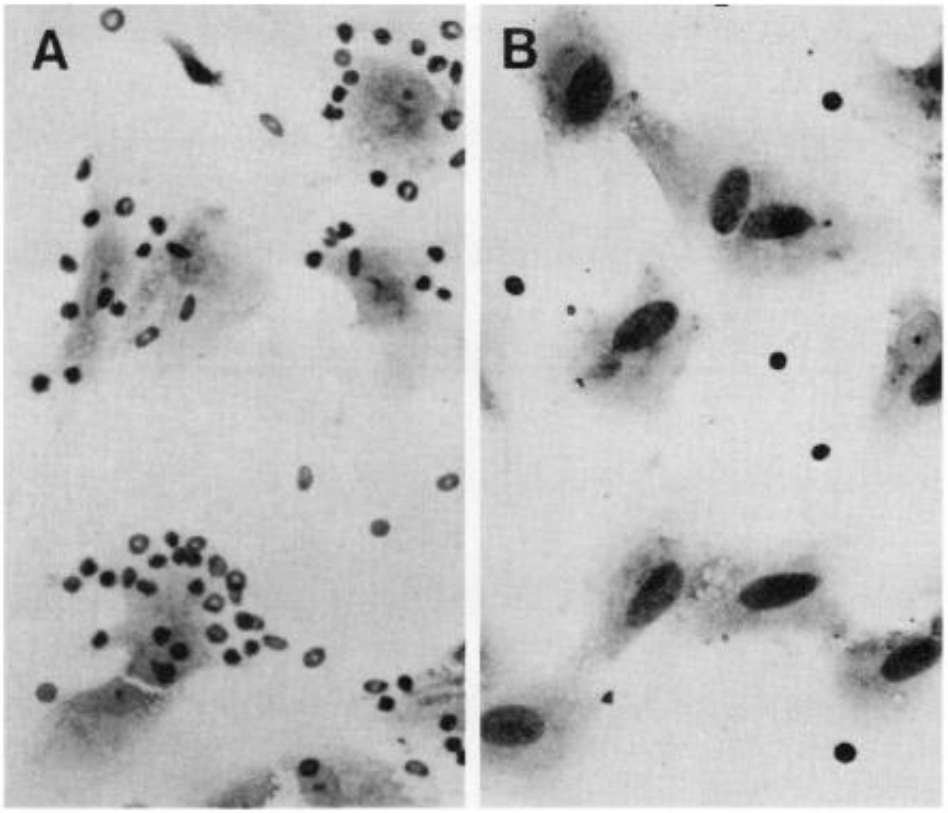
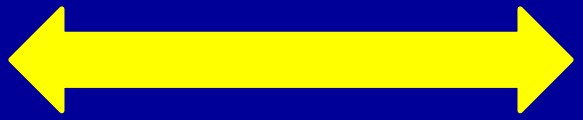
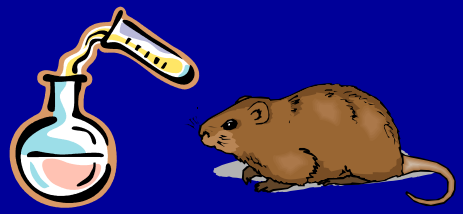


FIGURE 2 Distribution of RBC on subconfluent culture plates stained with Giemsa after the fifth plate wash. Sickle RBC distribute themselves in rosette-like clusters around endothelial cells (A), whereas normal RBC are present in fewer numbers and are randomly distributed (B). ×375.

Hebbel et al J. Clin. Invest. 65, 1980 157





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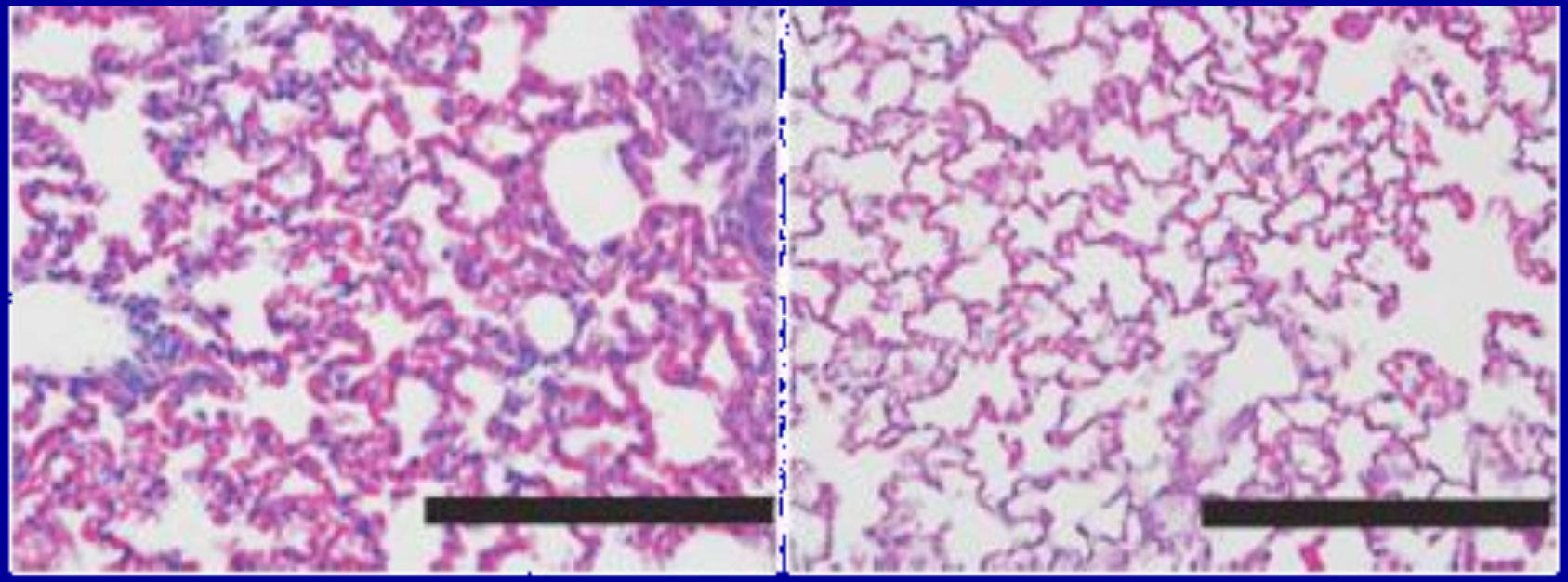
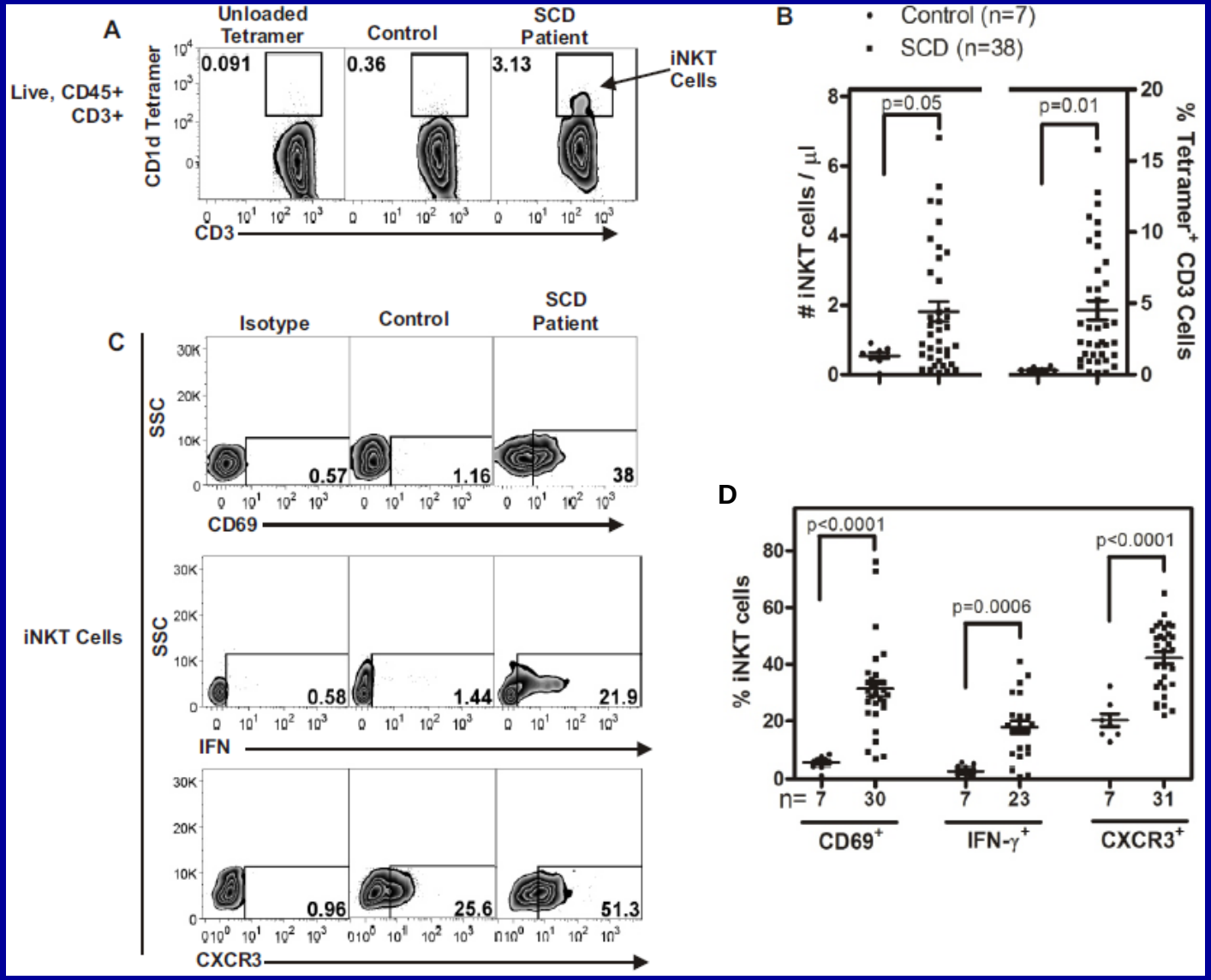
Hypoxia/Reoxygenation: A Mouse Model of ACS

NY1DD-Vehicle-H/R NY1DD-ATL146e-H/R

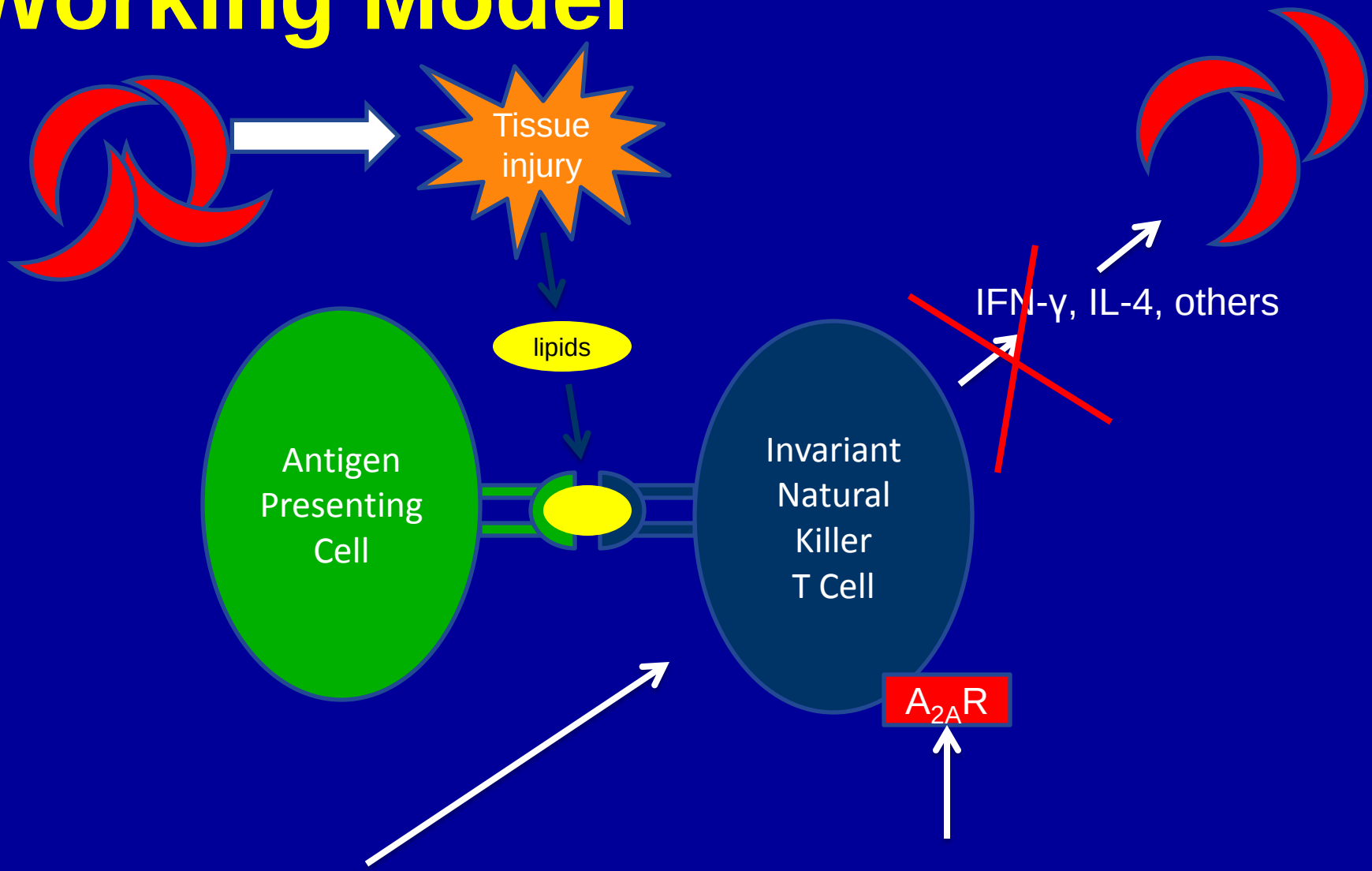


3 h hypoxia (8% O_2) 18h reperfusion (air)
A2A agonist started 3 h after reperfusion

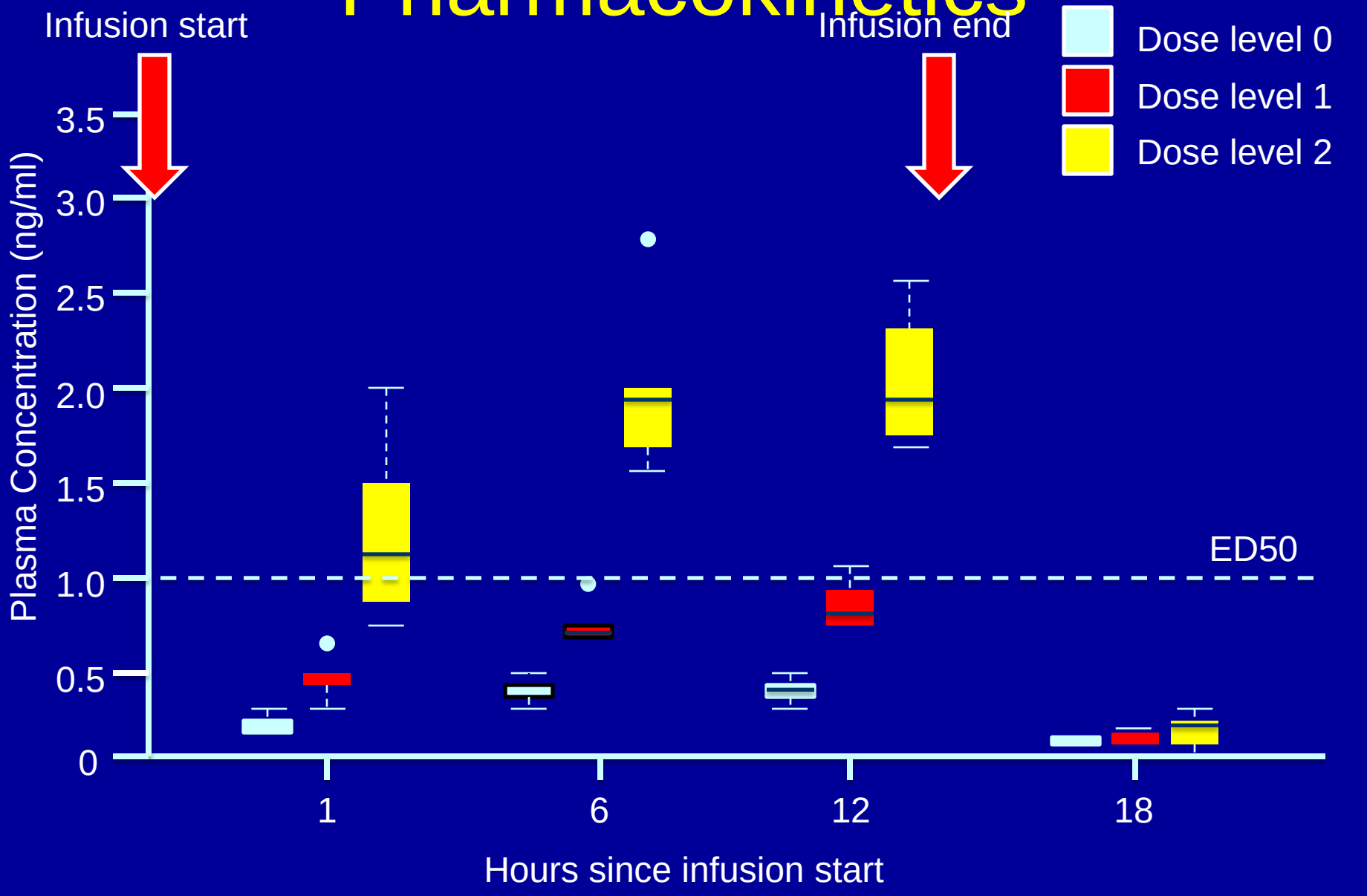
Human Blood


A
Unloaded Tetramer
Control
SCD Patient
Live, CD45+ CD3+
CD1d Tetramer
0.091
0.36
3.13
iNKT Cells
CD3
B
Control (n=7)
SCD (n=38)
p=0.05
p=0.01
# iNKT cells / µl
% Tetramer+ CD3 Cells
C
Isotype
Control
SCD Patient
iNKT Cells
SSC
0.57
1.16
38
CD69
0.58
1.44
21.9
IFN
0.96
25.6
51.3
CXCR3
D
% iNKT cells
p<0.0001
p=0.0006
p<0.0001
n= 7 30 7 23 7 31
CD69+
IFN-γ+
CXCR3+

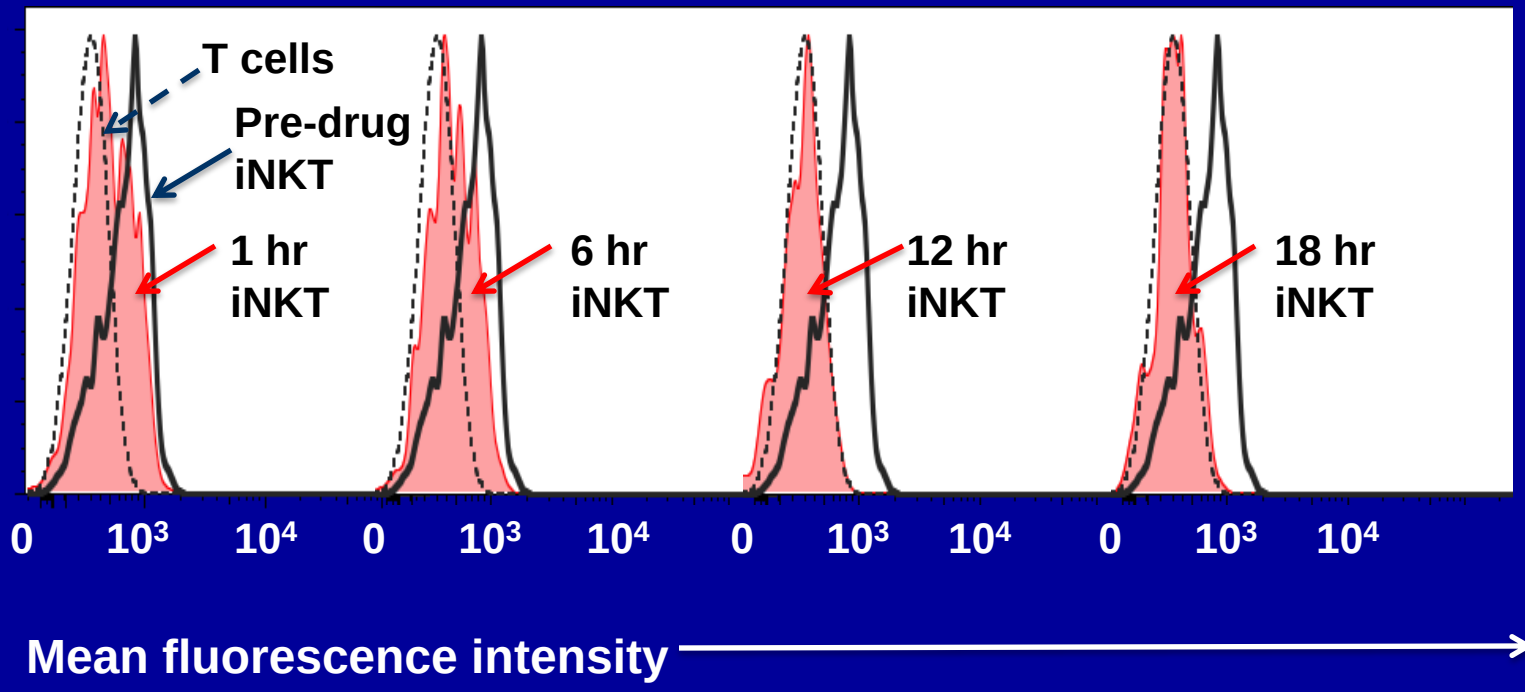
Working Model



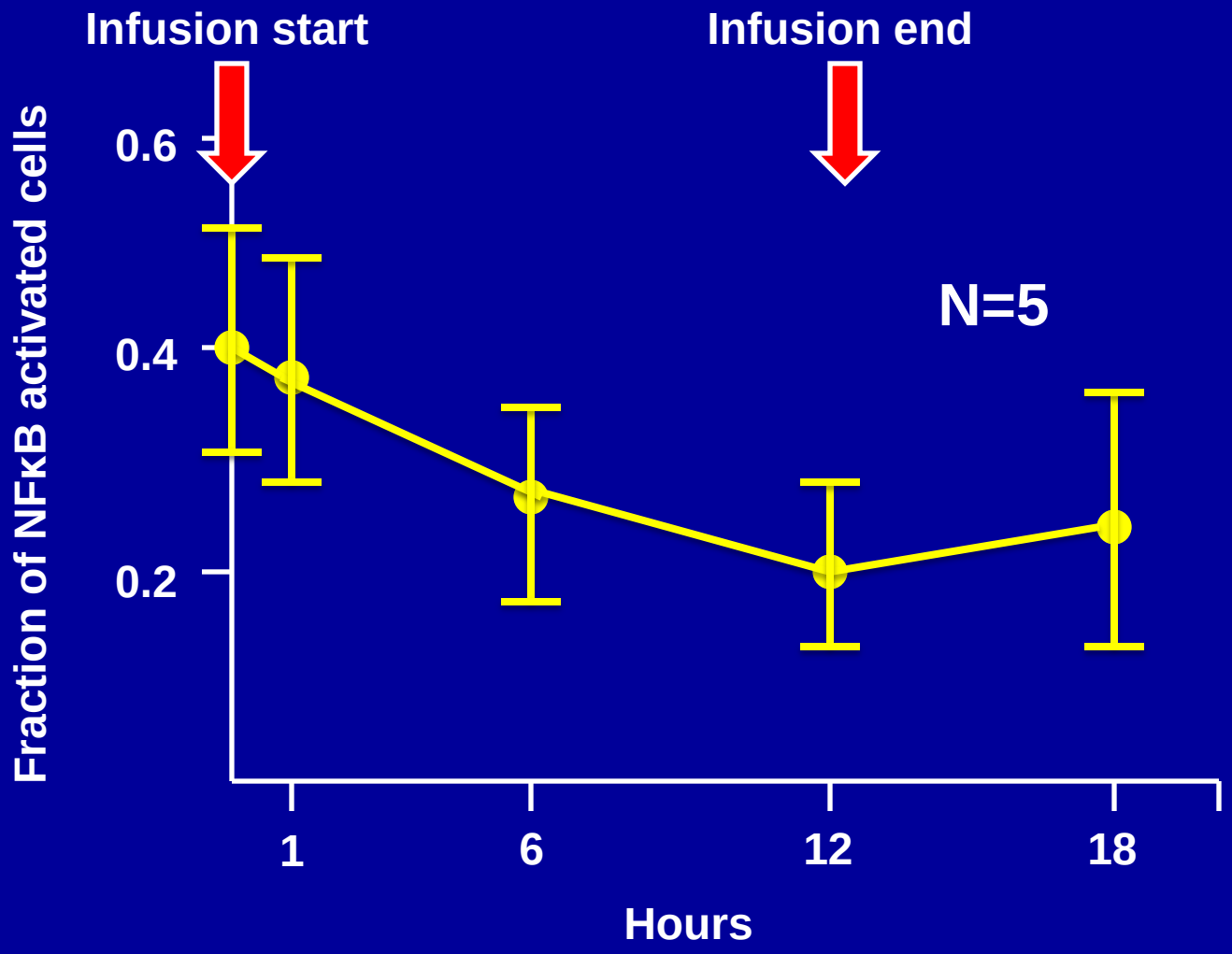
Regadenoson Pharmacokinetics



Subject 9: NFκB during infusion



NFκB+ Cells In Activated Gate: Dose Level 2



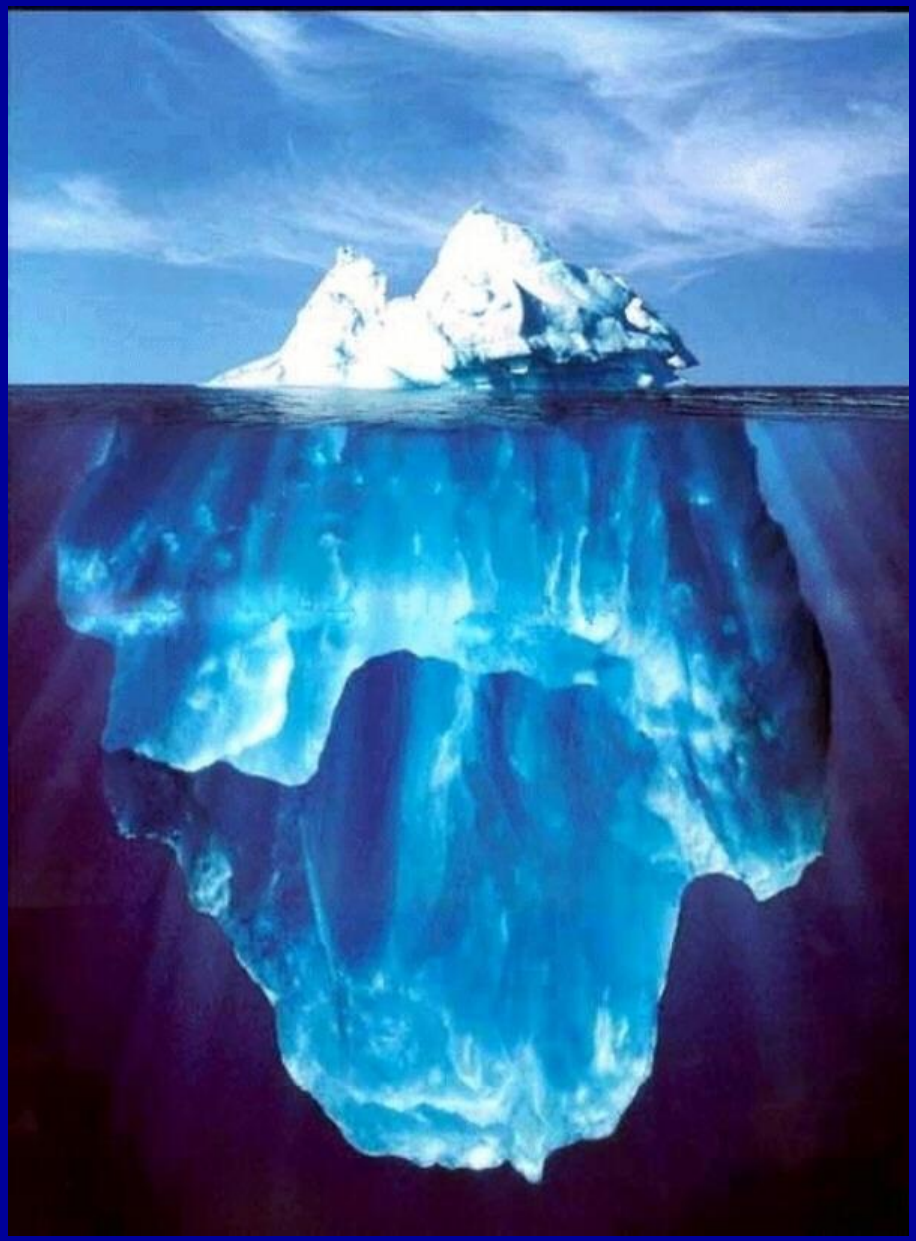
Proposed Future Studies

- Pilot Study of Regadenoson for the Treatment of Acute Chest Syndrome
  - Primary endpoint: Accrual
- Phase II Trial of Regadenoson for the Treatment of Acute Vaso-occlusive Episodes
  - Primary endpoint: Reduction in inflammatory biomarkers
- Anti-NKT cell monoclonal antibody

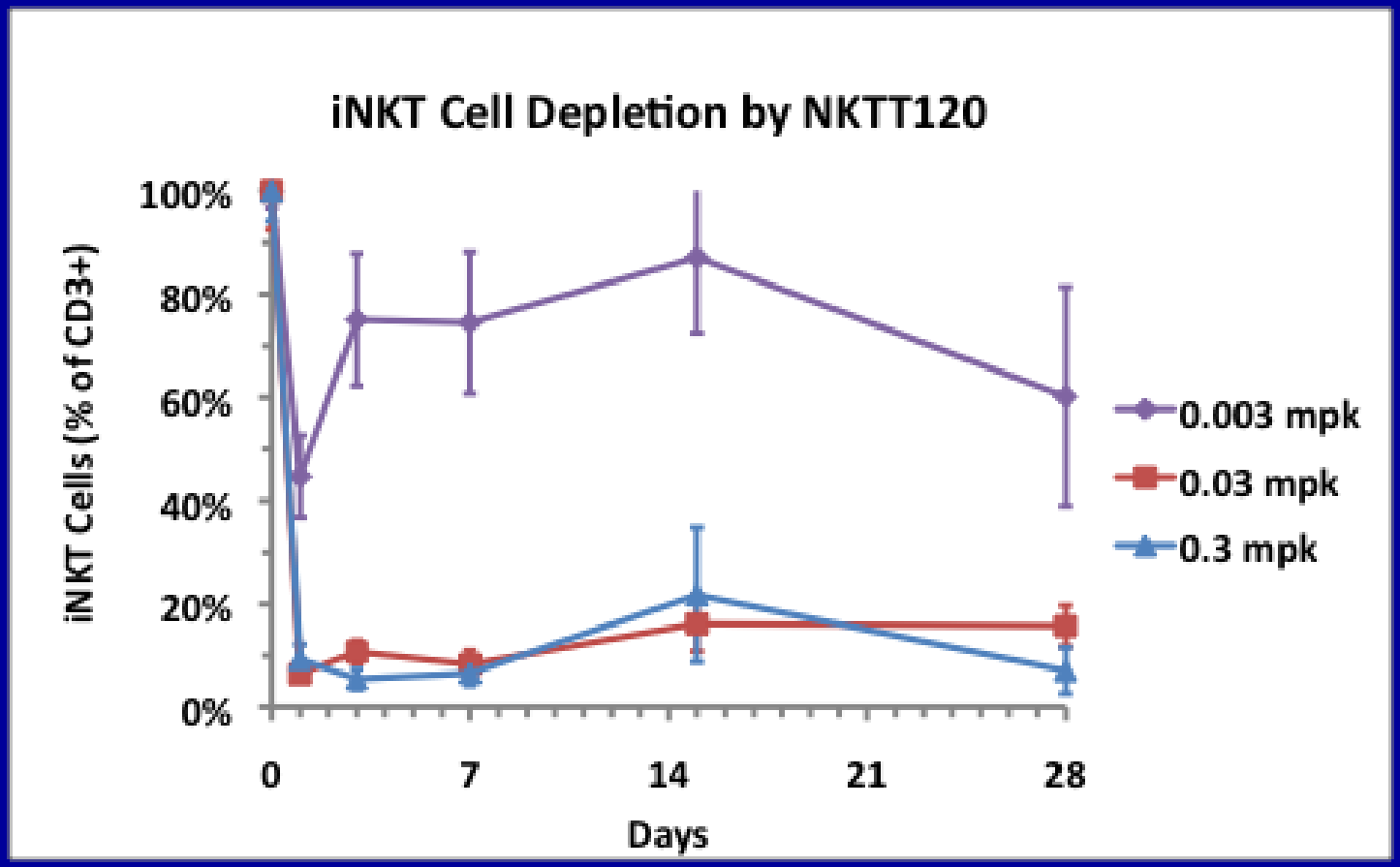
Acute and Chronic VOC

- Pain and ACS leading to hospitalization are the tips of the iceberg.
- Thirty percent of adults with SCD report pain on a daily basis.

Smith et al 2008



iNKT Cell Depletion by NKTT120 (Standard IgG1)



DNA Sequence Polymorphism

- Common SNPs may be biologically trivial or evolutionarily constrained and thereby important in some way.
- Rare variants associated with mendelian disorders are much more apt to be important so full DNA sequencing should be useful (up to a point)
- But “guilt by association” often leads to a dangerous conclusion (SNPs around CRP show big effects in heart disease but obviously have no causal relationship)

Exceptions Prove the Rule

- BCl11A discovered in a common SNP search related to fetal hemoglobin and has a big effect size (ten percent)
- Three other SNPs related to fetal hemoglobin bring the total effect size to near fifty percent
- Alpha thalassemia is also clearly associated with decreased severity
- Yet published and unpublished GWAS that evaluate severity do not reveal HbF associated SNPs or SNPs that might relate to alpha globin synthesis. Faint association with TGF beta suggesting that inflammation might be important

WILL GENOME SEQUENCING REVEAL THERAPEUTIC LEADS IN SCD?

- “Unbiased” research=fishing expedition
- Hypothesis should precede not follow technology application in science
- “Guilt by association” is dangerous in medicine as well as law
- Poorly crafted GWAS are worse than no studies at all
- Best to compare lowest five percent and highest five percent for each severity category

SCD ATTACK POINTS

- Increase delay time with a small molecule without changing O2 affinity (cyanate which does change affinity and is neurotoxic) or a stapled peptide that would compete with Val 6
- Increase or introduce a hemoglobin (HbF or Hb Korle Bu) with hydrophobic binding to val 6 in HbS, prolongs delay time and inhibits stacking of polymers by forming hybrids (hydroxyurea and/or BCL11A inhibition)
- Hematopoietic stem cell transplant
- Gene therapy by excision and replacement by a non S allele
- Suppression of inflammation and ischemia reperfusion injury
- Prenatal diagnosis
- Control asthma