Novel approaches to desensitization

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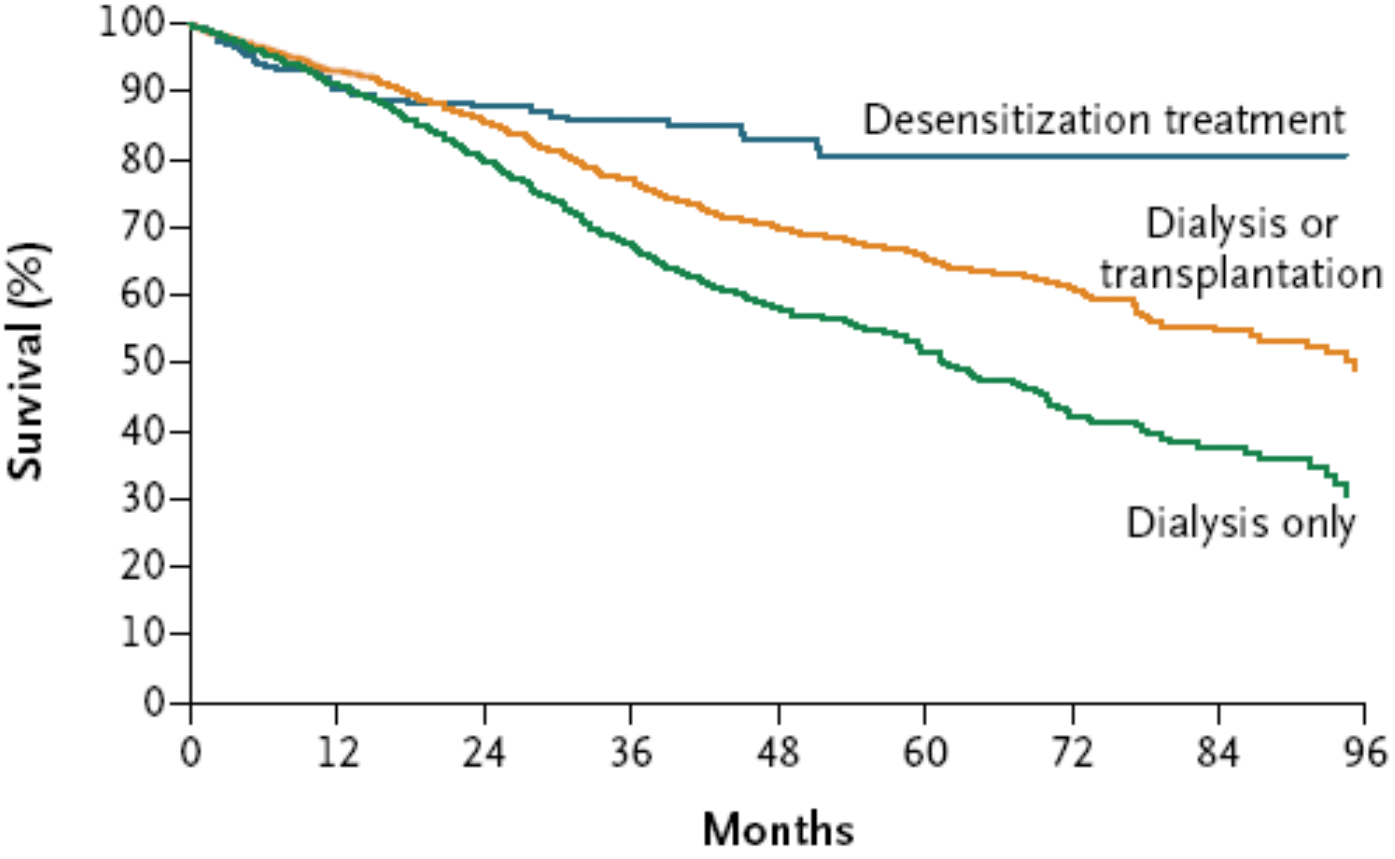
INTRODUCTION

- Transplant wait lists continue to grow in parallel with increased demand for organs and limited donor supply pool.
- Sensitized patients represent a particular challenge .
- Increasing number of patients on mechanical circulatory support.
- Pre- transplant sensitization is associated with longer wait time to transplant and increased risk of rejection after transplant .

History

- Desensitization therapies started to emerge in the 1980's .
- Donor specific blood transfusions were performed for HLA desensitization with limited success
- There was more success with transplantation during this time period with techniques employing a combination of plasma exchange (PLEX) & splenectomy .
- HLA antibody desensitization with intravenous immunoglobulin (IVIG) was first reported in the mid-1990's and ushered in a new era of transplantation .
- New immunomodulatory therapies



No. at Risk

Desensitization treatment	210	170	143	110	75	58	42	28	14
Dual therapy	1027	854	688	497	321	230	157	96	41
Dialysis only	1012	822	626	419	250	159	93	54	17

Procedure	dialysis	Uncomplicated Tx	Complicated Tx	Functioning graft
Cost /year ($)	84550	29920	106000	18000

Desensitized arm		Dialysis arm
desensitization	28090	238667
transplant	92799	
Continuing dialysis	84639	
Treatment of rejection -/+ Return to dialysis	14386	
Total cost	219914	238667
Saving	**18753 (7.9% of 3 years dialysis)**	

Most importantly

Tx was associated with a 14.7–17.6% increased survival compared to dialysis

Definition of a highly sensitized patient

PRA value of 85%, defined in the complement-dependent cytotoxicity (CDC) assay.

However the PRA value of the same serum determined by different laboratories could even vary between 5% and 80% while the assignment of HLA antibody specificities in CDC was more reproducible .

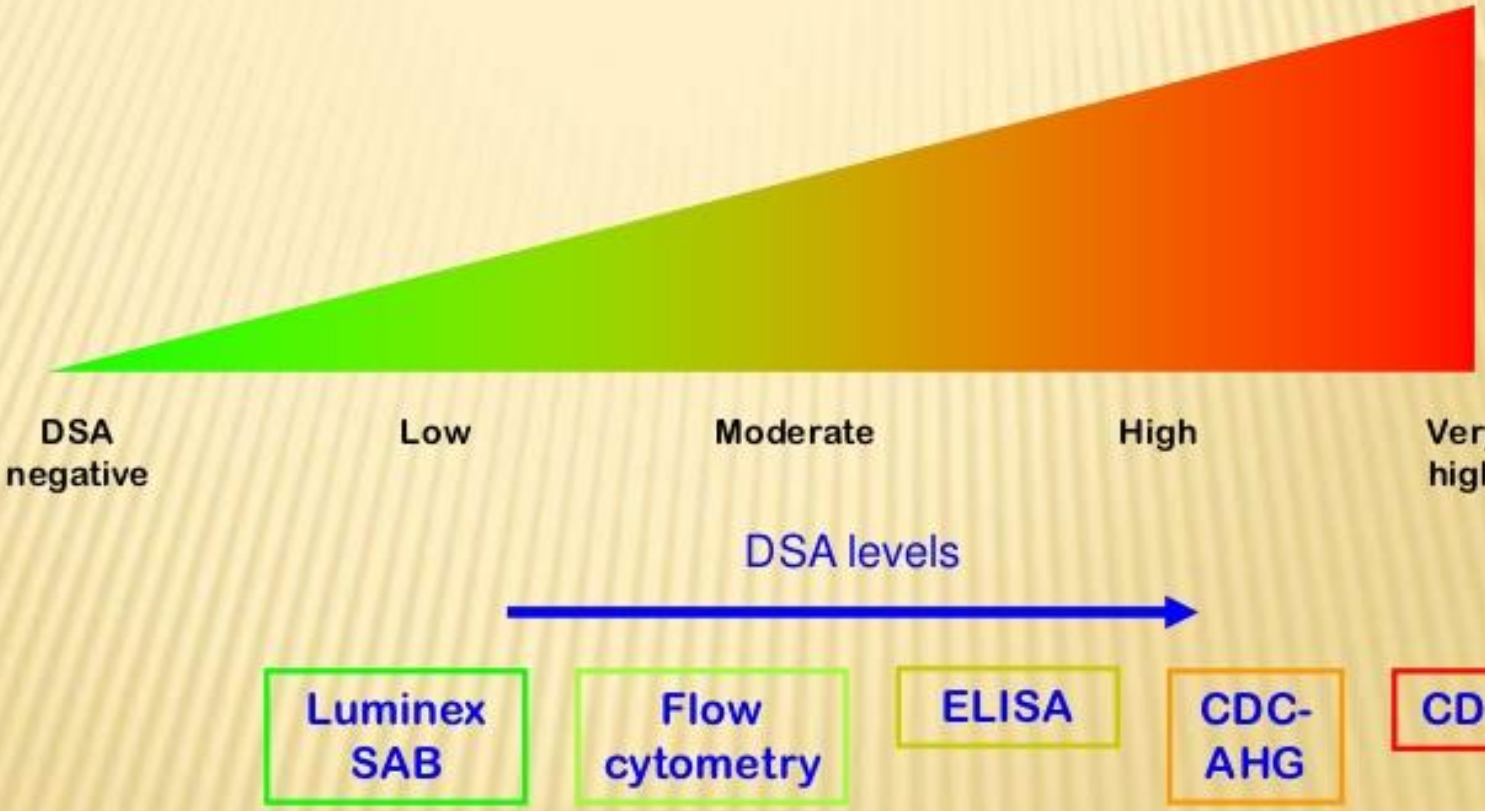
A better definition of the degree of sensitization is based on the specificities of the HLA antibodies of a patient in relation to the frequencies of the target antigens in the donor population, (% PRA = % population reactive antibodies(calculated PRA= cPRA)

Theoretical number of match runs to have a 95% chance of finding an acceptable donor

cPRA, %	number of match
10	2
20	2
30	3
40	4
50	5
60	6
70	9
80	14
85	19
90	29
95	59
99	300
99.5	600
99.9	3000
99.99	30,000
99.999	300,000

Technique / Level of antibody	Luminex	FCXM	CDC
Low	Positive	Negative	Negative
Moderate	Positive	Positive	Negative
High	Positive	Positive	Positive

Sensitivity of DSA identification methods



Level of antibody by flow cytometric crossmatch (FCXM)

negative	**<130 MCS for B cell <70 MCS for T-cell FCMX (after pronase digestion)**
weak	**<5000 MFI**
moderate	**5,000–10,000 MFI**
strong	**>10,000 MFI**

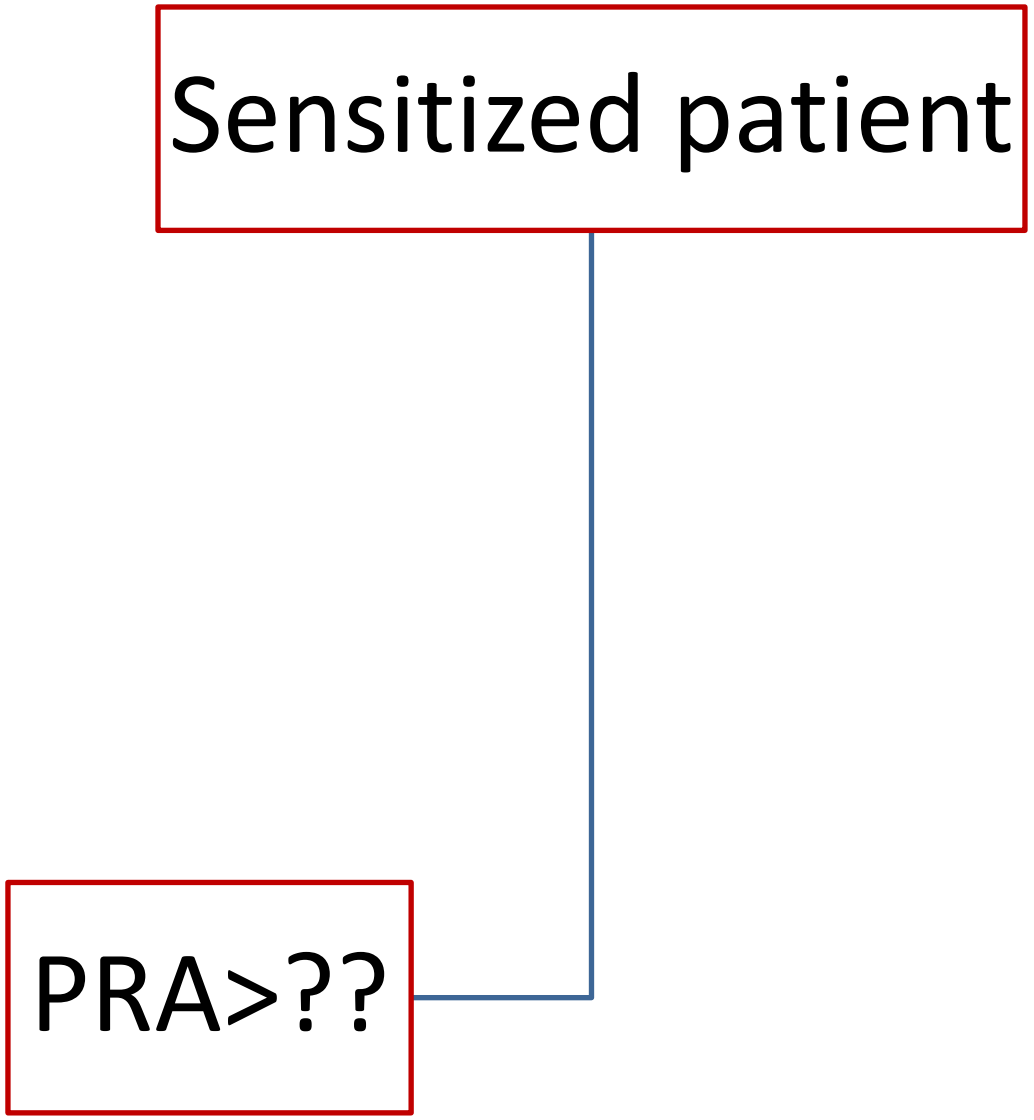
MCS = mean channel shift
MFI = mean fluorescence intensity

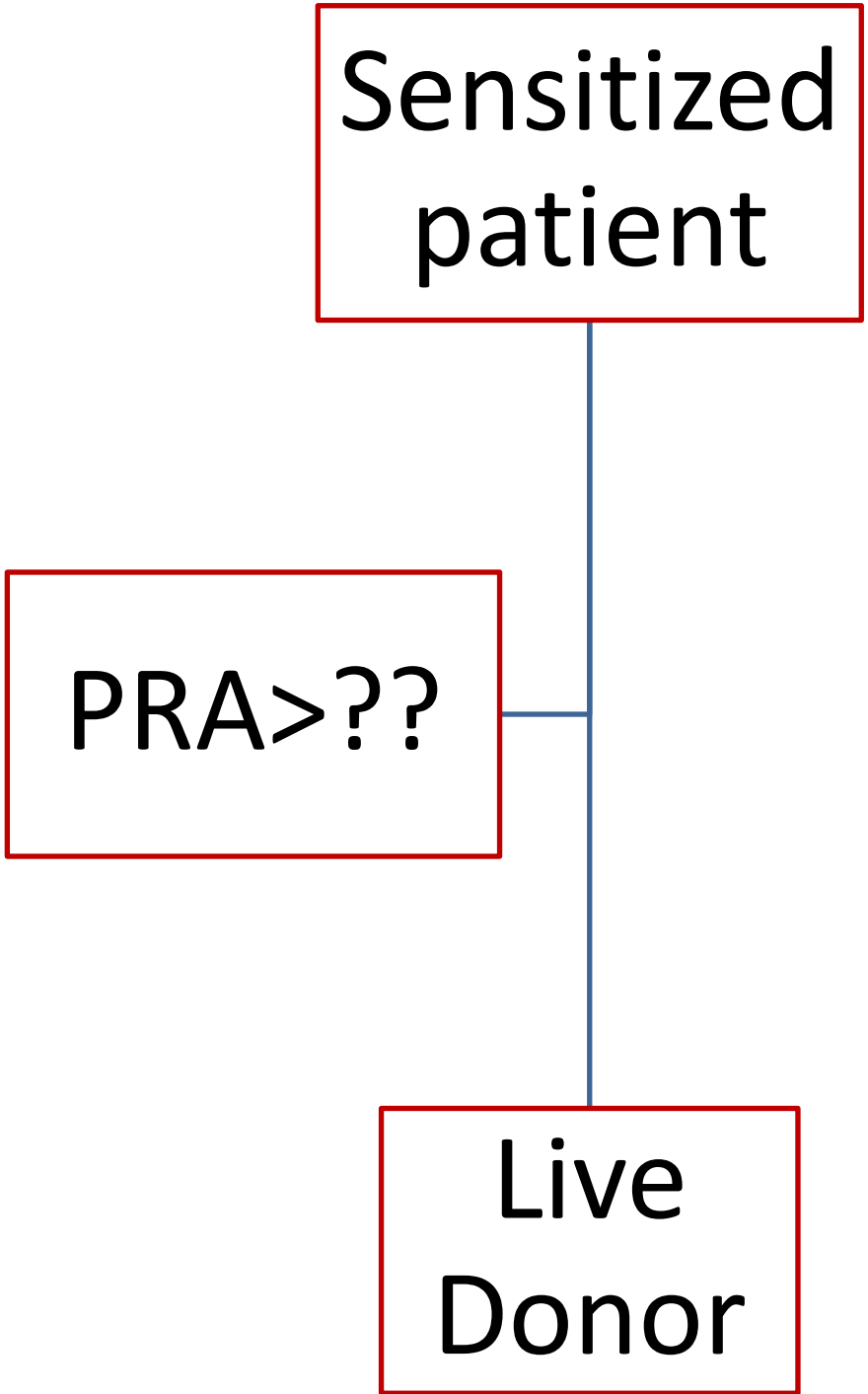
DSA-RIS (relative intensity scale)	points
No DSA	0
Each weak DSA (MFI < 5,000)	2
Moderate DSA (MFI 5,000–10,000)	5
Each strong DSA (MFI > 10,000)	10

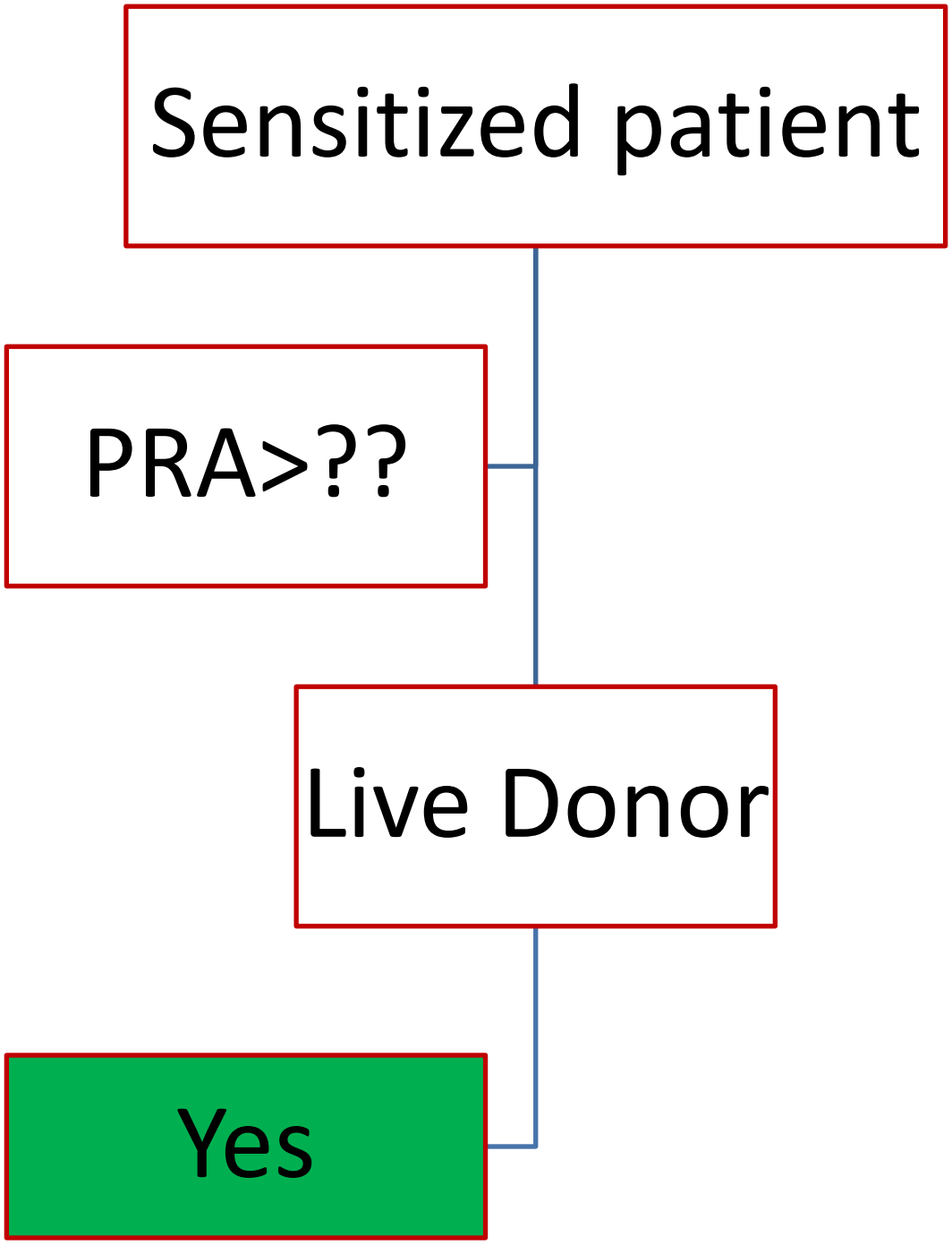
Successful desensitization

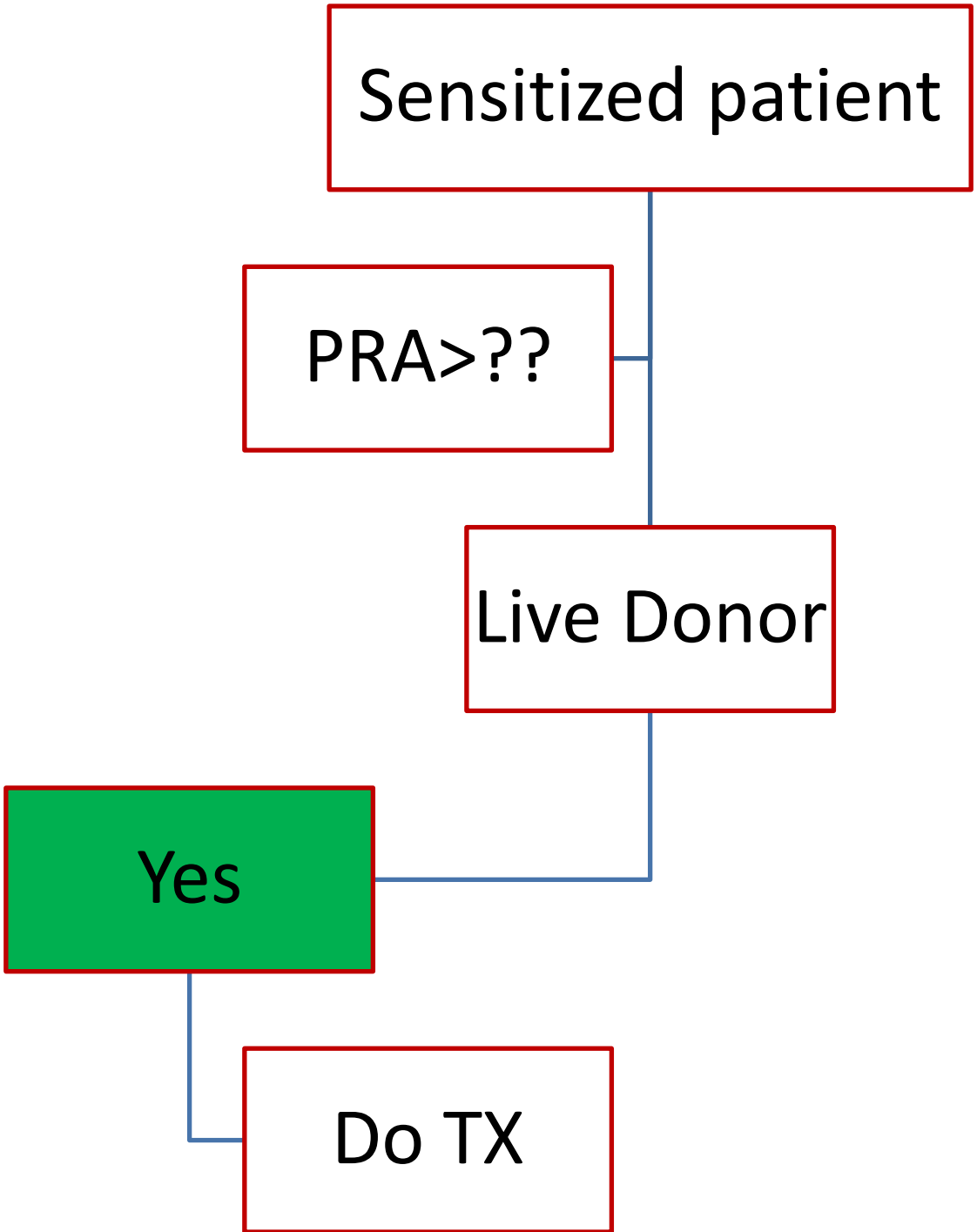
- Negative CDC <1:2 dilution
- FCMX(T&B): MCS < 225
- DSA RIS score <17
- Negative CMX : B flow < 100 or pronase < 130 MCS
- T flow < 50 or pronase < 70 MCS

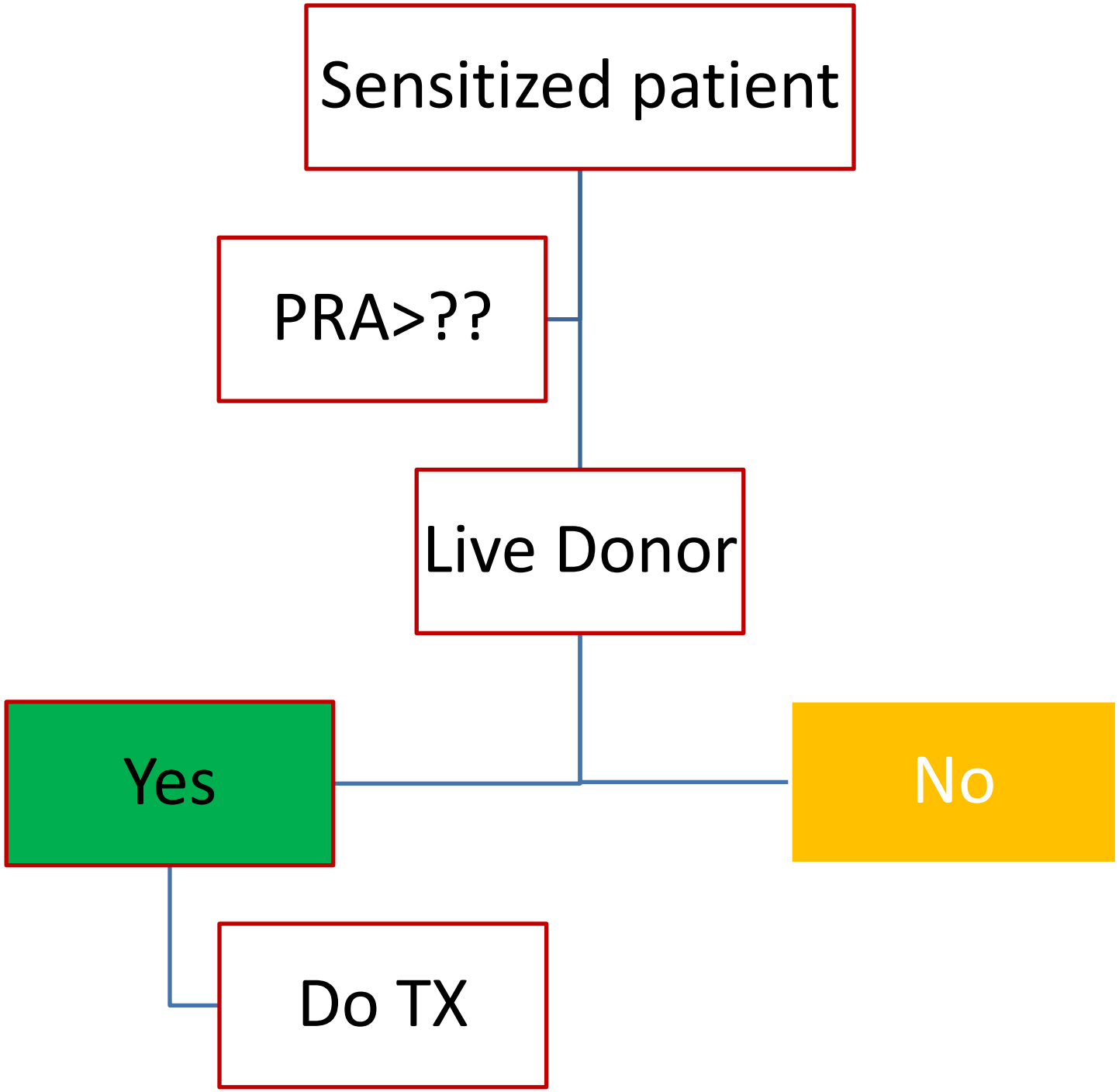
Sensitized patient

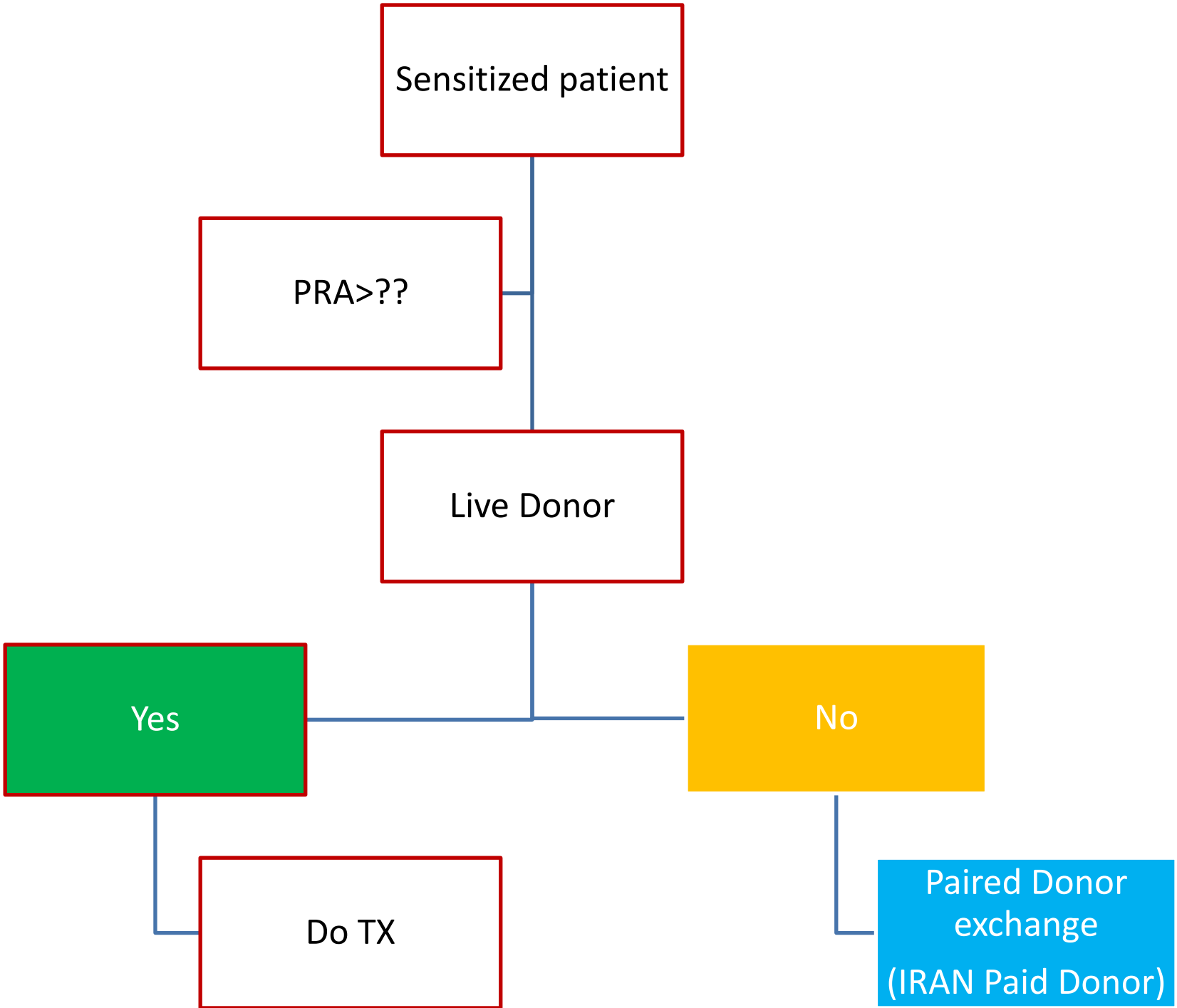

Sensitized patient
PRA>??

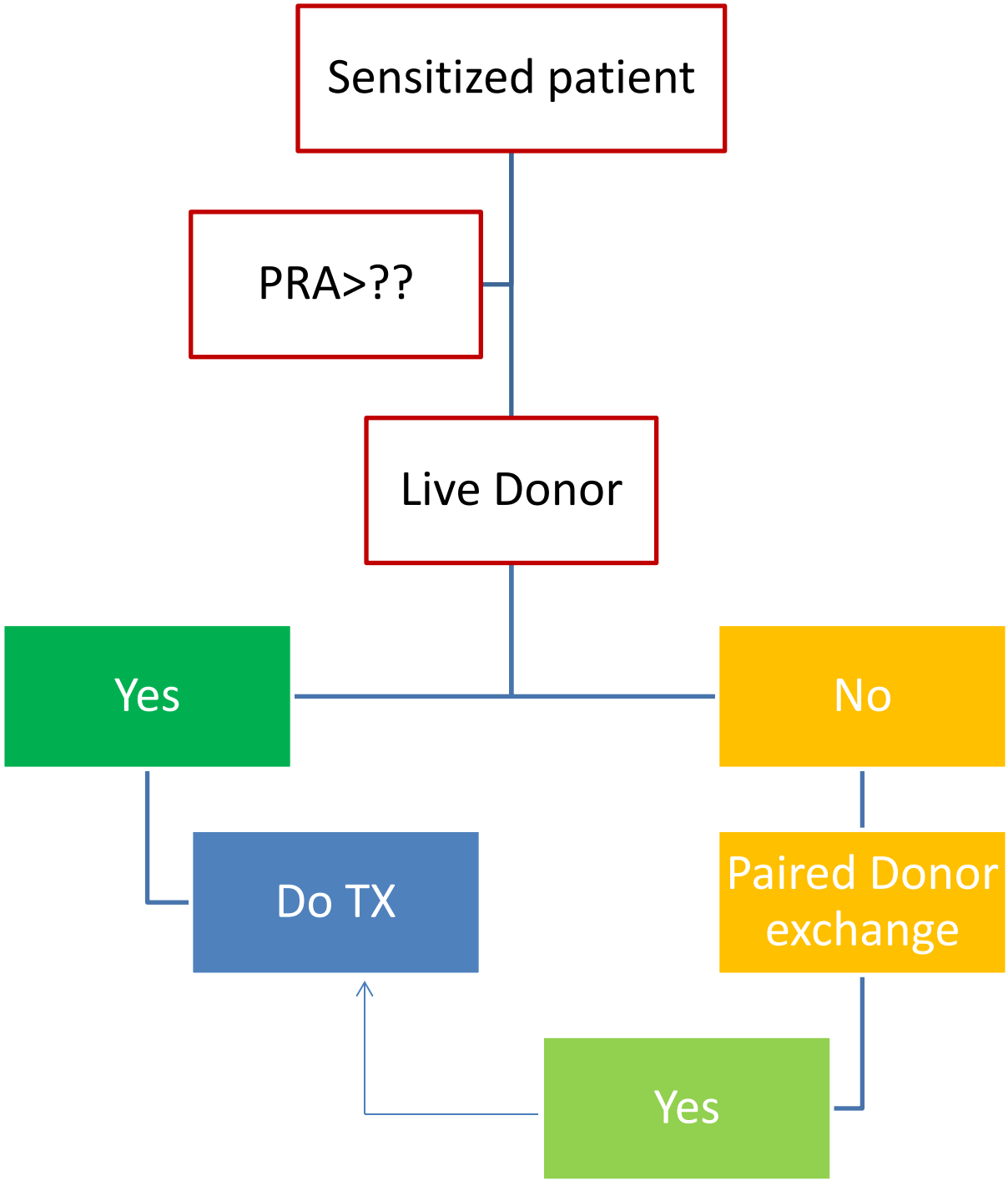

Sensitized patient
PRA>??
Live Donor

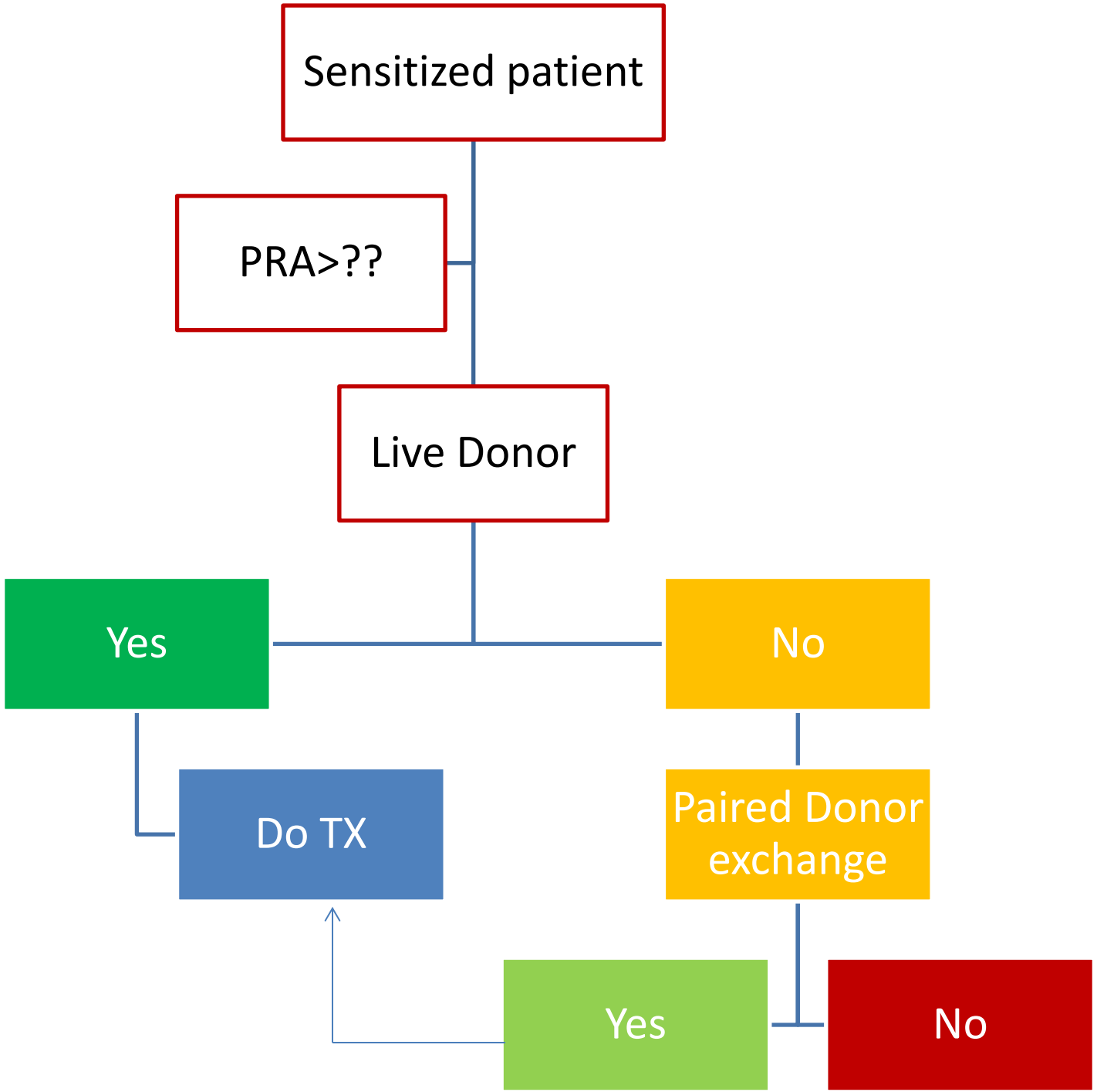

Sensitized patient
PRA>??
Live Donor
Yes

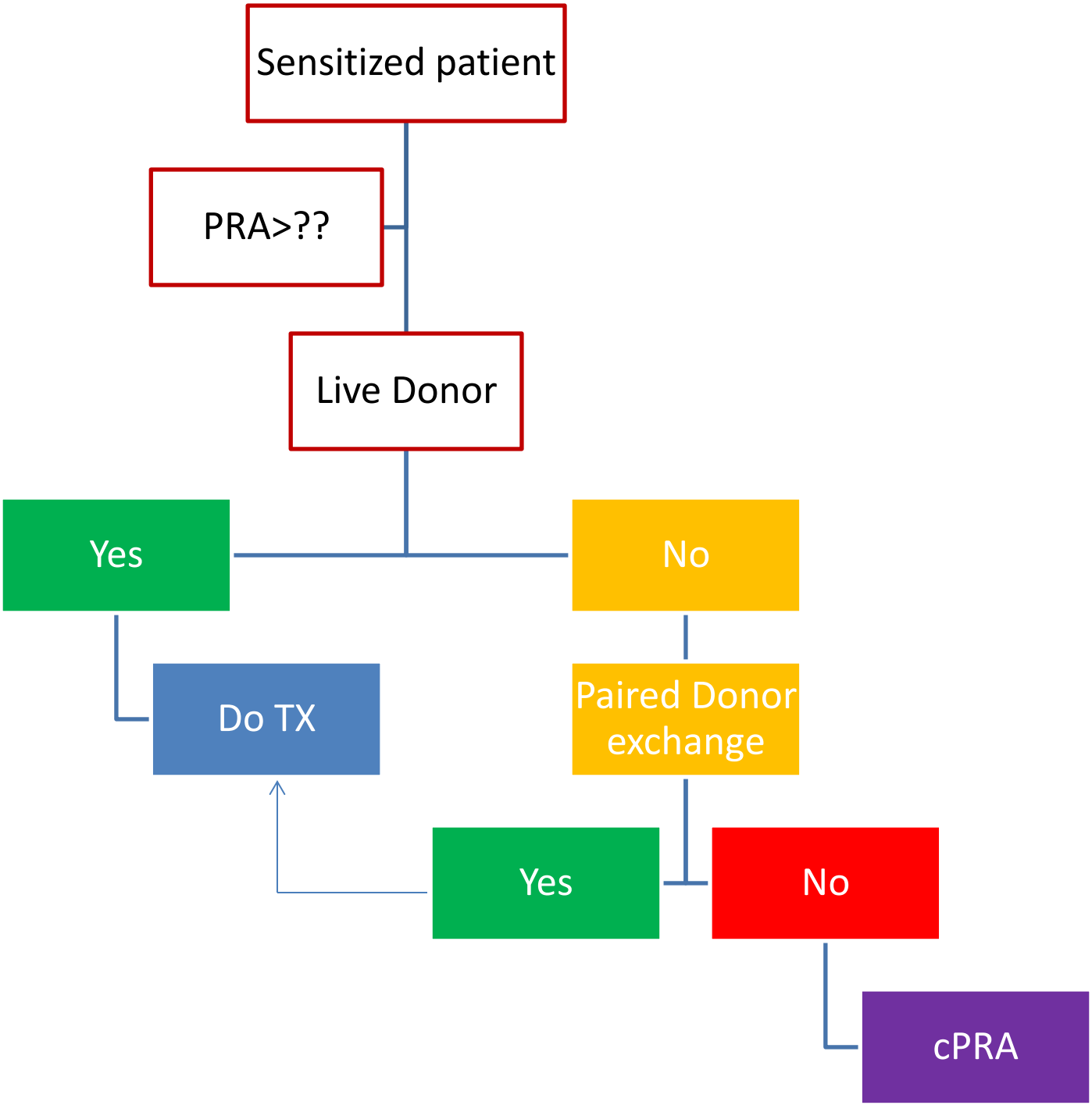

Sensitized patient
PRA>??
Live Donor
Yes
Do TX

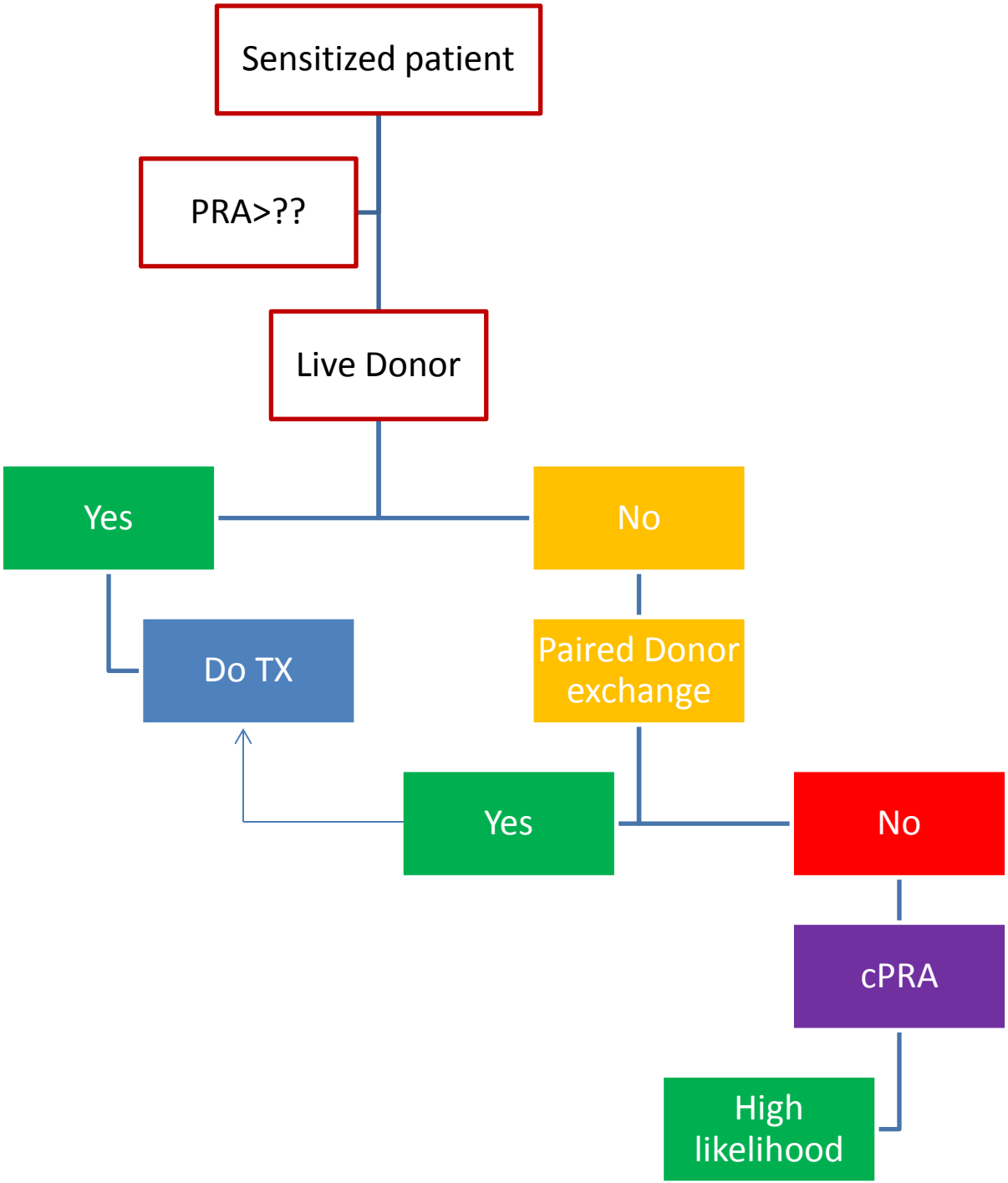

Sensitized patient
PRA>??
Live Donor
Yes
No
Do TX

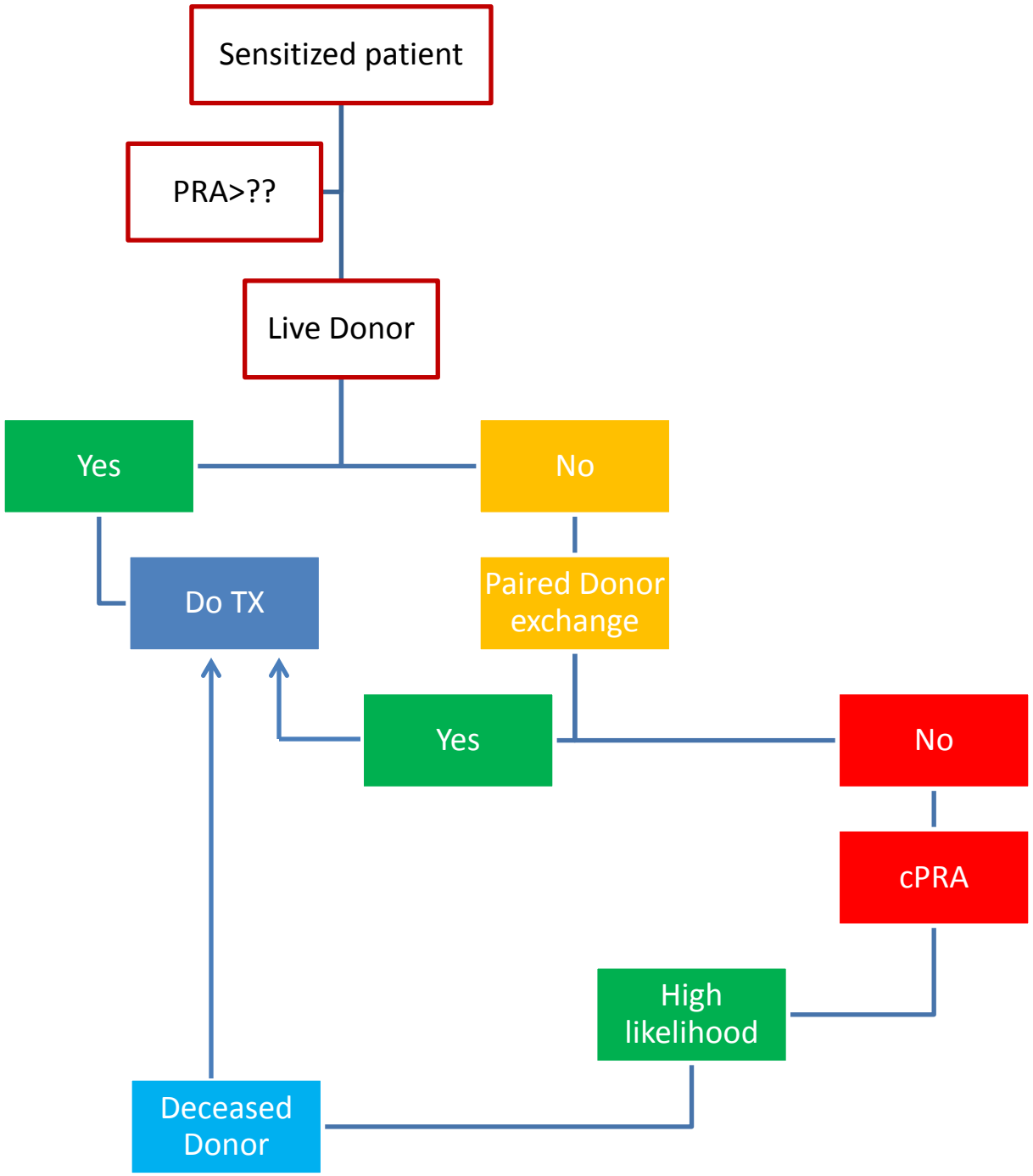

Sensitized patient
PRA>??
Live Donor
Yes
No
Do TX
Paired Donor exchange
(IRAN Paid Donor)

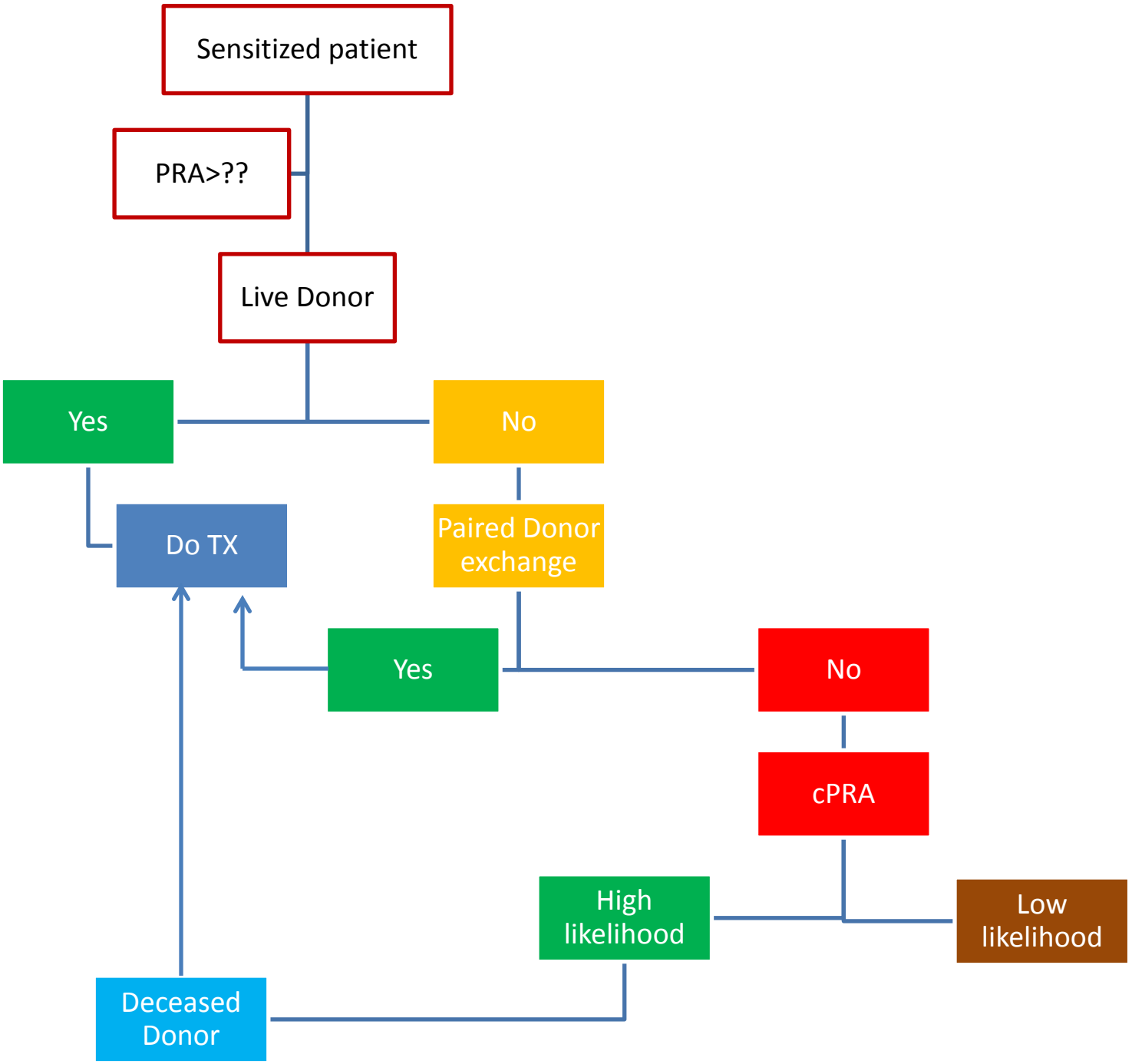

Sensitized patient
PRA>??
Live Donor
Yes
No
Do TX
Paired Donor exchange
Yes

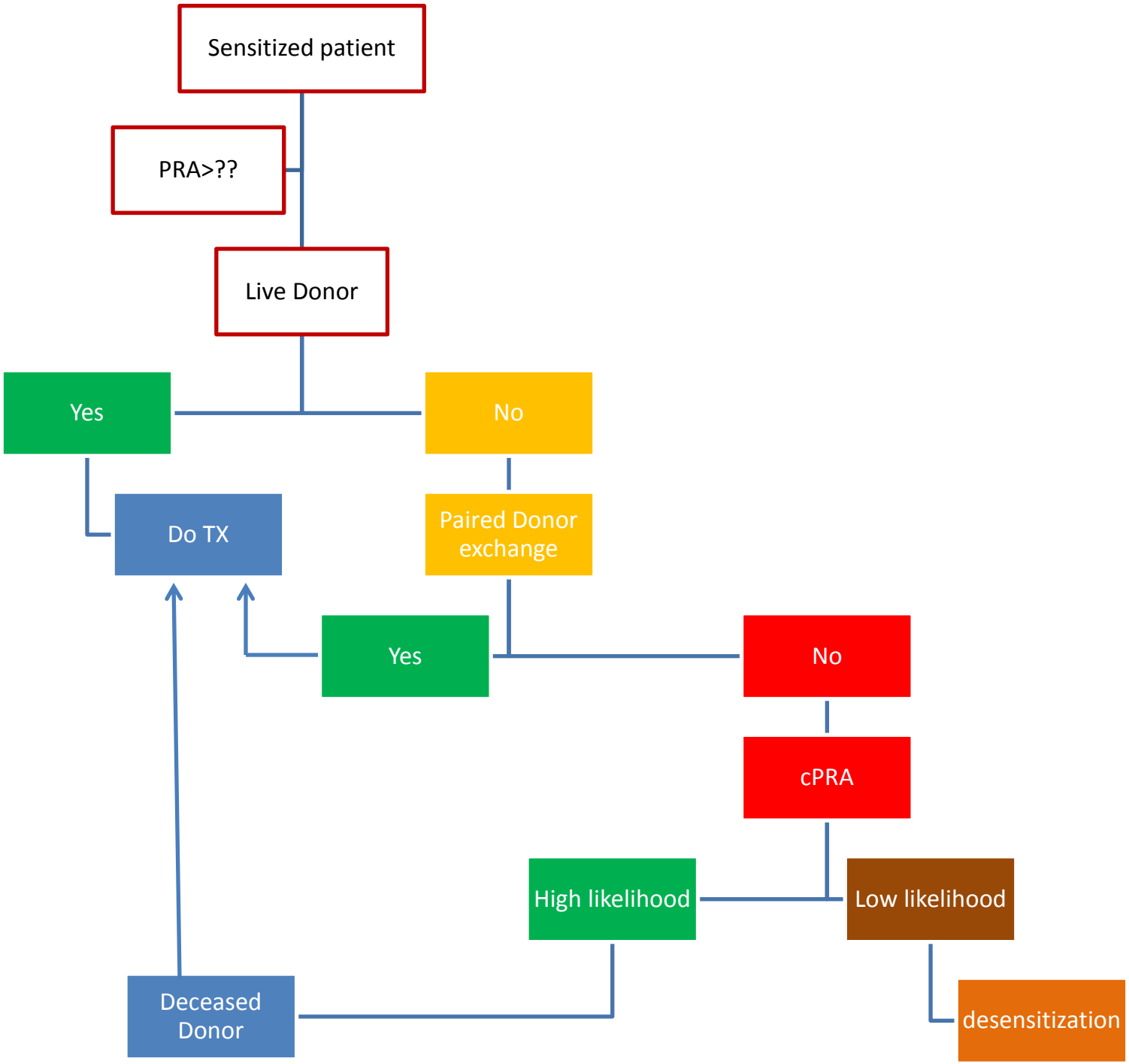

Sensitized patient
PRA>??
Live Donor
Yes
No
Do TX
Paired Donor exchange
Yes
No


Sensitized patient
PRA>??
Live Donor
Yes
No
Do TX
Paired Donor exchange
Yes
No
cPRA


Sensitized patient
PRA>??
Live Donor
Yes
No
Do TX
Paired Donor exchange
Yes
No
cPRA
High likelihood


Sensitized patient
PRA>??
Live Donor
Yes
No
Do TX
Paired Donor exchange
Yes
No
cPRA
High likelihood
Deceased Donor


Sensitized patient
PRA>??
Live Donor
Yes
No
Do TX
Paired Donor exchange
Yes
No
cPRA
High likelihood
Low likelihood
Deceased Donor


Sensitized patient
PRA>??
Live Donor
Yes
No
Do TX
Paired Donor exchange
Yes
No
cPRA
High likelihood
Low likelihood
Deceased Donor
desensitization

Basics of desensitization

1- Removal of antibodies by PP or IA

2- Inhibition of antibody production

A: Anti-B cell agents: rituximab (anti-CD20)

B: Plasma cell inhibitors: bortezemib (proteosome inhibitor)

3- Inhibition of complement cascade: eculizumab (anti-C5a)

4- IVIG has multiple effects on different immune pathways:

A: Neutralization of circulating anti-HLA antibodies through anti-idiotypic antibodies

B: Inhibition of complement activation by binding C3b and C4b and neutralization of C3a and C5a

C: Blockage of immune activation and enhancing the clearance of anti-HLA antibodies by competing for activating FcγRs

D: Inhibits the expression CD19 on activated B cells and induces apoptosis of B cells

E: Induces the expression of FcγIIB, which is a negative regulatory receptor on immune cells

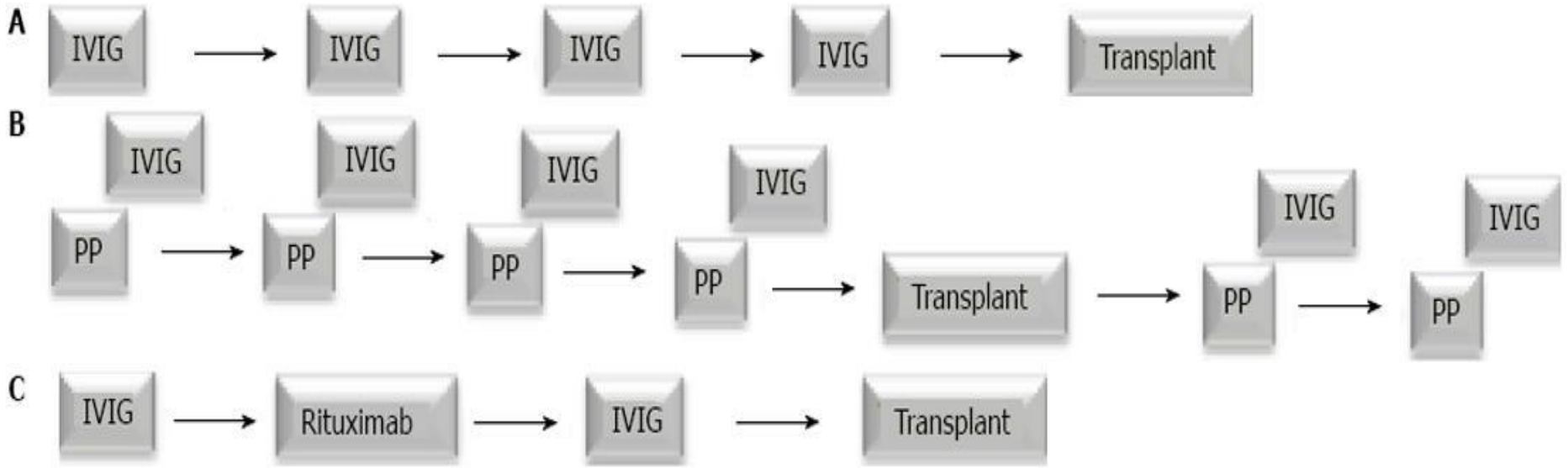
F: Inhibitory effects on cellular immune responses and nonspecific inhibitory effects on the immune system by binding to Fcγ receptors on macrophages, neutrophils , platelets, mast cells, and natural killer cells and inhibiting cytokine, chemokine , adhesion molecules, and endothelial cell activity

5- Splenectomy

(removes a major source of lymphocytes, including antibody-secreting B cells, B cell precursor cells, and plasma cells)

Modern approaches to incompatible kidney transplantation

- IVIG the cornerstone of all desensitization protocols



A: The NIH : (IVIG) in four monthly doses followed by a living or deceased donor transplant once an acceptable crossmatch was achieved ;
B: Johns Hopkins University used a combination plasmapheresis (PP) with low-dose cytomegalovirus immune globulin following each PP session. The number and frequency of the PP sessions is dependent on the donor specific antibody titer .
C: A modified protocol combining IVIG and rituximab was developed at Cedars-Sinai Medical Center. Two doses of IVIG are administered one month apart with one dose of rituximab given in between .

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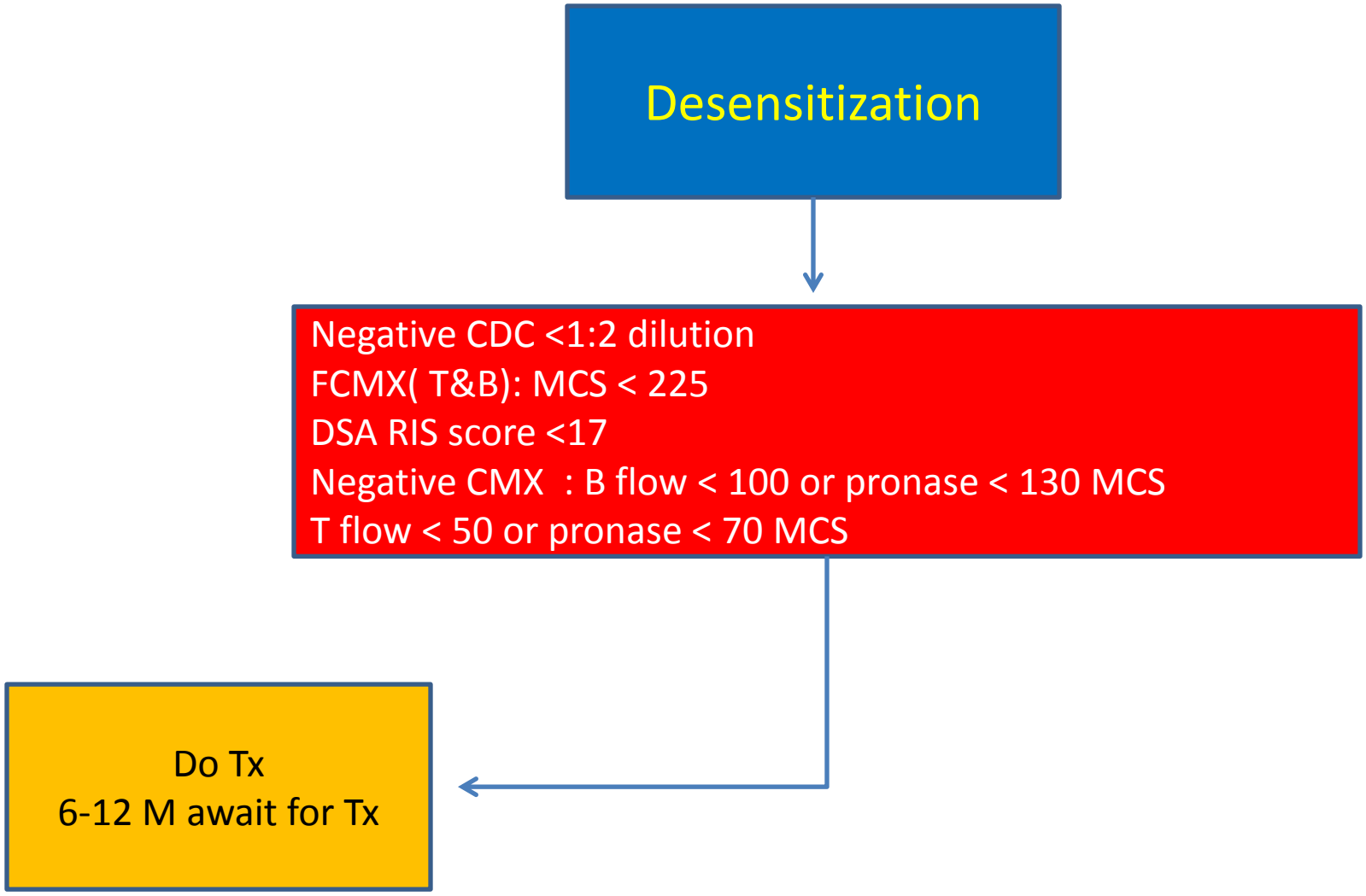
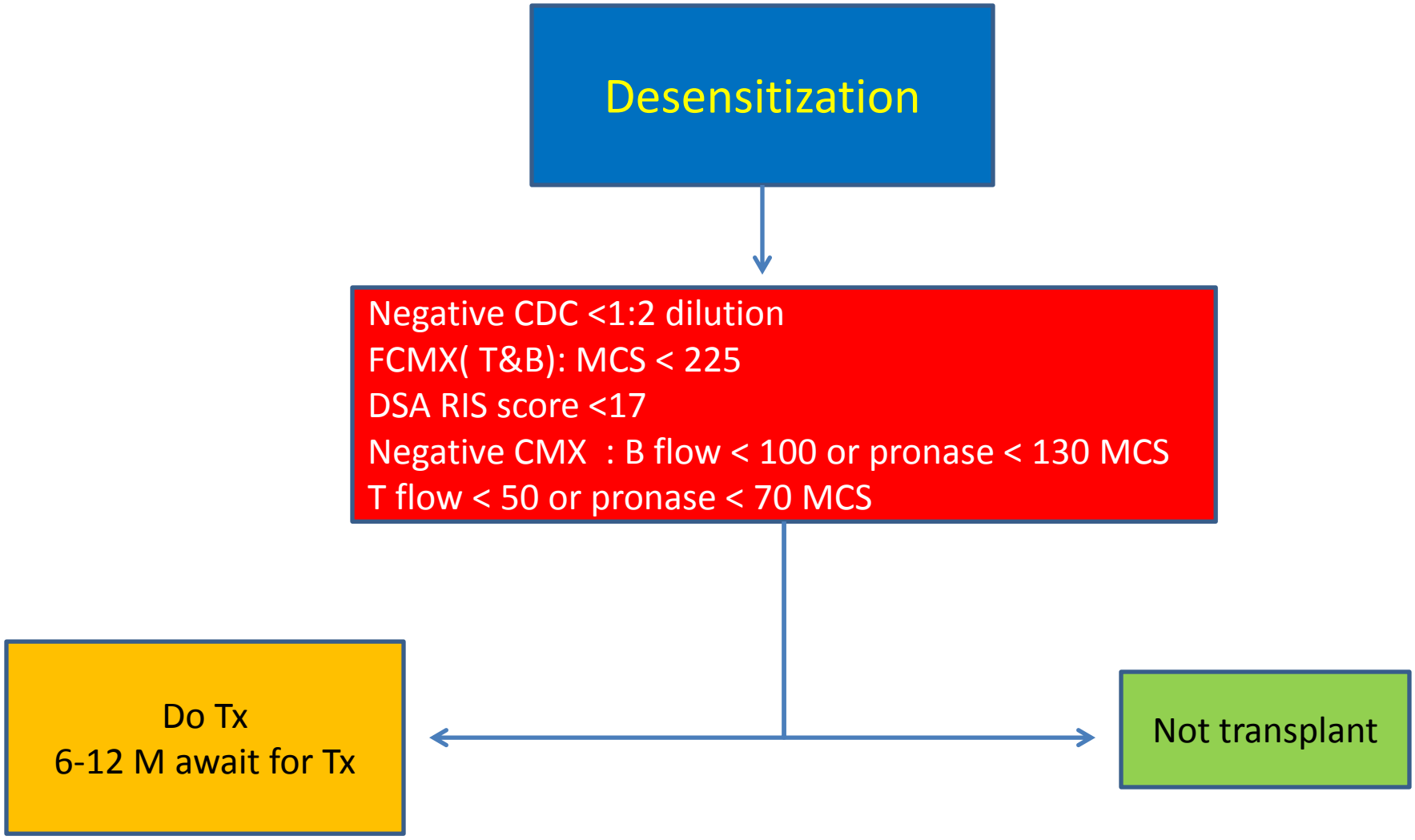
Successful desensitization

Desensitization


Desensitization
Negative CDC <1:2 dilution
FCMX(T&B): MCS < 225
DSA RIS score <17
Negative CMX : B flow < 100 or pronase < 130 MCS
T flow < 50 or pronase < 70 MCS
Do Tx
6-12 M await for Tx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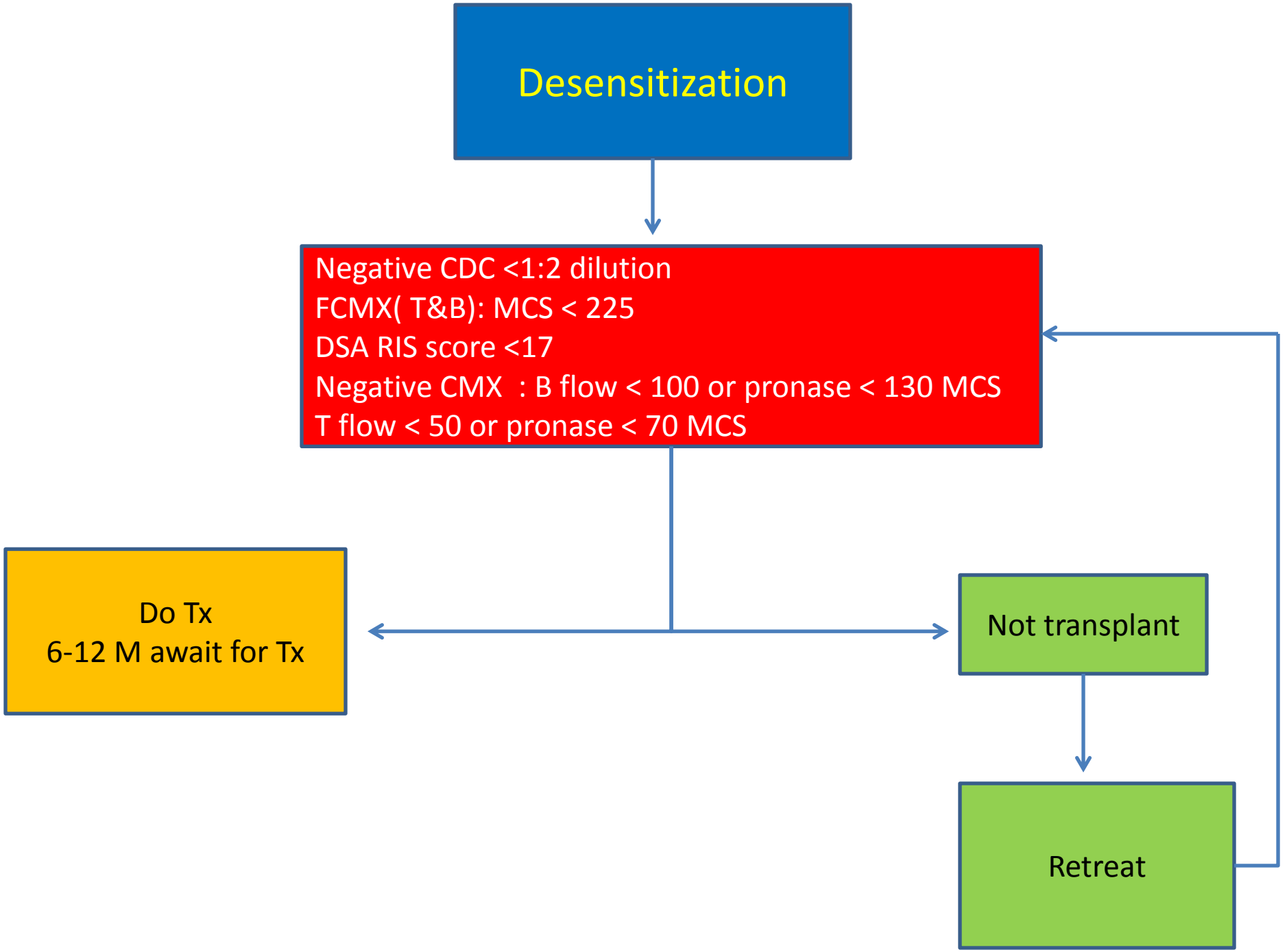
DSA RIS score <17
Negative CMX : B flow < 100 or pronase < 130 MCS
T flow < 50 or pronase < 70 MCS
Do Tx
6-12 M await for Tx
Not transplant


Desensitization
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FCMX(T&B): MCS < 225
DSA RIS score <17
Negative CMX : B flow < 100 or pronase < 130 MCS
T flow < 50 or pronase < 70 MCS
Do Tx
6-12 M await for Tx
Not transplant
Retreat

Future approaches

- The ongoing development of biologic agents particularly for the treatment of rheumatologic diseases may provide new avenues of exploration for desensitization .
- All of these agents modulate B cell activity .
- Epratuzumab targets CD22 on B cells and effectively modulates their activity. It has shown promise in patients with systemic lupus erythematosus (SLE) .
- Belimumab, an antibody directed against B lymphocyte stimulator (BLyS) was recently approved for SLE and also has B cell modulator effects *via* inhibition of B cell proliferation .
- Atacicept is currently under study in SLE and acts as a soluble receptor for the B cell proliferation cytokines BLyS and a proliferation-inducing ligand (APRIL) thereby neutralizing their activity. A decrease in total IgG levels has been demonstrated in early phase studies .
- Tocilizumab is a monoclonal antibody directed against the receptor for interleukin-6, a potent inflammatory cytokine. It is currently approved for rheumatoid arthritis and leads to reductions in IgG and inflammatory responses. It was shown in to modulate the development of DSA in a mouse model of allosensitization.

Novel therapeutic agents for kidney transplantation

Medication (generic/trade name)	Mechanism of action	Potential use in kidney transplant (clinical trial reference)
Tocilizumab (®Actemra)	receptor 6 Soluble and membrane-bound IL- antagonist	•Desensitization (NCT01594424) •Treatment of antibody-mediated rejection
Belimumab (Benlysta ®)	Prevents B-lymphocyte stimulator protein from stimulating B-cell activation and differentiation	•Desensitization (NCT01025193, terminated) •Prevention of kidney transplant rejection (NCT01536379)
C1 esterase inhibitor (Berinert®)	s of the 1r and C1inhibitor inactivates both C1 C complement pathway	•Prevention of antibody-mediated rejection (NCT01134510) •Delayed graft function and ischemic reperfusion injury (NCT02134314)
C5 inhibitor (Eculizumab®)	b 5a and C5inhibitor preventing cleavage to C5 C , terminal 9b-5preventing formation of C complement complex	•Desensitization (NCT01567085) •Delayed graft function and ischemic reperfusion injury (NCT01756508, NCT0919346) •Kidney Transplantation in Catastrophic Antiphospholipid Antibody Syndrome (NCT01029587) •Antibody-mediated rejection (NCT01327573),(NCT02113891)
IgG Endopeptidase (Ides ®)	Cleavage of all four classes of Human IgG	•Desensitization (NCT02224820)