**Table 1 Available assays evaluating alloreactive serological memory**

|  |  |  |  |
| --- | --- | --- | --- |
| **Method** | **Antigen detection system** | **Advantages** | **Limitations** |
| Complement-dependent cytotoxicity | Donor lymphocytes  Rabbit complement | PPV+++ of Hyperacute rejection | Low sensitivity for low.level antibodies  FP results by non-HLA or auto-antibodies |
| Flow cytometry | Donor lymphocytes  Fluorescent labelled antibody to T/B cells and to IgG | More sensitive than CDC  Detects low level DSA  Predictive of early AMR | Positivity may be due to nonspecific antibody confirmed by a positive auto-XM  In absence of DSA by SAB, a positive FC XM is not predictive of reaction |
| ELISA | HLA molecules from platelet donors or EBV-transformed cells on a microtiter plate | First assays to use capture HLA proteins, enabling testing without donor cells | Low sensitivity and specificity |
| Bead-based assays on Luminex | HLA purified antigen on plastic beads  Fluorescent labelled antibody to IgG. Beads can have a mix or individual HLA (SAB) | More sensitivity and specificity than CDC and FC in sensitized patients  Less FP than ELISA, especially for class II antibody | Interpretation requires expertise  Significant variations between laboratories and kits  FP reactions for denatured conformation of HLA on bead surface not correlated with AMR |

AMR, antigen-mediated reaction; CDC complement dependent cytotoxicity; DSA, donor –specific antibodies; EBV, Epstein Barr virus; ELISA, enzyme linked immunosorbent assay; FC, flow cytometry; FP, false positive; HLA, human leukocyte antigen; Ig, immunoglobulin; MFI, mean fluorescent intensity; PPV, positive predictive value; PRA, panel of reactive antibodies; SAB, single-antigen bead; XM, crossmatch

**Table 2 Summary of pharmacologic options**

|  |  |
| --- | --- |
| **DRUG** | **Mechanism of action** |
| Rituximab | Murine/human mAb binding CD20 present on pre-B and mature lymphocytes |
| Daratumumab | IgG1k humanized mAb directed against CD38 |
| Eculizumab | mAb binding protein C5, inhibiting cleavage to C5a and C5b and formation of membrane attack complex C5b-9 |
| C1 esterase inhibitor | Inhibits activation of complement and intrinsic coagulation pathway |
| Tocilizumab | Recombinant humanized antihuman IL6 receptor mAb. Binds both soluble and membrane bound IL6R |
| Clazakizumab | Genetically engineered, humanized IgG1 mAb; IL6 ligand inhibition |
| IgG degrading enzyme of S. pyogenes. IdeS | Enzyme that cleaves all 4 IgG antibodies into F(ab)2 and Fc |

**Table 3 DSA monitoring for the first year after transplantation with different treatments**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | rATG alone  (N= 10) | rATG + RTX  (N = 10) | rATG + Bortezomib  (N = 10) | rATG + RTX + Bortezomib  (N = 10) | p-Value |
| Development of de novo DSA | 3/10 (30%) | 3/10 (30%) | 1/10 (10%) | 3/10 (30%) | 0.70 |
| Time to de novo iDSA appearance (days) | 38 | 101 | 10 | 185 | 0.33 |
| Time to de novo iDSA peak (days) | 38 | 323 | 11 | 186 | 0.13 |
| De novo iDSA level at the end of 1-year follow-up (MFI) | 0 | 0 | 0 | 0 | 0.68 |
| Number of patients with increase in de novo iDSA | 0/3 | 1/3 | 0/1 | 0/3 | 1.00 |
| Number of patients with decrease in de novo iDSA | 3/3 | 2/3 | 1/1 | 3/3 | 1.00 |

**Table 4 Results of 4 patients with ABMR treated by anti CD38**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Transplant** | **Sensitization** | **Treatment** | **AntiCD38 use** | **Evolution** |
| Heart + kidney | Immunized:  Preformed DSA | Steroid Pulse +ATG+IVIG+RTX+ Eculizumab | 16 mg/kg. infusion for 8 weeks | Clinical good  HLA dramatic decline |
| Kidney | Immunized: preformed DSA | No treatment added to the standard of care | 16 mg/kg for 8 weeks + 1 monthly infusion for 9 months | DSA undetectable  Stabilization of renal function |
| Kidney | Immunized:  AB0i (Anti A) | Steroid pulses  ATG  Immunoadsorption  Eculizumab | 16 mg/kg for 6 weeks infusions | Kidney function recovered  Reduction in anti A titer |
| Heart | Immunized:  Preformed DSA | Steroid pulses  Immunoadsorption | 16 mg /kg infusion for 8 weeks + 1 infusion monthly for 9 months | Improvement  Only slight reduction in DSA |