CNI-freie Immunsuppression: Modell für die Zukunft?

Langzeitergebnisse und Sicherheit innovativer Immunsuppression nach Nierentransplantation

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Disclosures

- Dr. Arns and his institution have received scientific grants and consultation honoraria from Novartis, Astellas, Roche, BMS and Pfizer.
Contents

● What have we learned from recent studies?
● The DSA concept
● The immunological and non-immunological risk
● The comorbidity concept
Considerable improvements have been made in acute rejection and short-term graft survival but…

![Graph showing acute rejection and graft survival over time with medications used in different years.](image)

- **80–90%**
- **40–50%**
- **5–20%**

**Year of transplant**

- Azathioprine
- Prednisone
- CsA
- MMF
- Basiliximab
- Everolimus
- Sirolimus

**Acute rejection**

- ~60%
- 40–50%
- 5–20%

**1-year graft survival**

- 80–90%

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**CsA, cyclosporine; ATG, anti-thymocyte globulin; MMF, mycophenolate mofetil.**

... long-term renal allograft survival has not improved...

Cumulative graft t½ by transplant year (forecasted Kaplan-Meier estimates)

Deceased donor kidney transplant attrition rates in the USA (n=164,480)

... neither has long-term patient survival

Patient 5-year survival 1994–2007
Recipients of a first deceased-donor kidney

ANZDATA registry 2012 report.
Cardiovascular disease and malignancy are the leading causes of death with functioning graft

Causes of death with functioning graft according to the ANZDATA registry 2007–2011

- Cardiovascular disease (CVD): 34%
- Malignancy: 31%
- Infection: 19%
- Miscellaneous: 10%
- Social: 7%

Data from 2007–2011; n=1071 patients in Australia; Data from 2007–2011; n=852 patients in Australia

Kidney function at Month 12 is associated independently with graft failure

Relationship between eGFR and graft failure over 10-years of follow-up (Cox proportional hazard adjusted for multiple covariates)

Hazard ratio (95% Confidence Interval) vs. 12-month eGFR, mL/min/1.73m²

- eGFR <50 mL/min/1.73m² was associated with proportionally lower graft survival, death-censored graft failure and death with function

eGFR, estimated glomerular filtration rate.
Patients with eGFR levels <45 mL/min/1.73m² have an increased risk of CVD and mortality

Hazard ratios of CVD and mortality (n=3676)

Reduced risk of CVD/death

Increased risk of CVD/death

≥75

1.04

1.07

60–74

Reference

Reference

45–60

0.88

1.02

30–45

1.39

1.34

<30

1.85

2.05

Hazard ratio (95% CI)

0.5

1

1.5

2

2.5

3

3.5

eGFR, mL/min/1.73m²

CI, confidence interval; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate.
Even patients with high 1-year eGFR can have progressive graft failure

Graft failure rate was higher among the high-eGFR progressors than the low eGFR group (34% vs 19%, P<0.0021)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>%</th>
<th>#</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-NP*</td>
<td>553</td>
<td>1%</td>
<td>7</td>
</tr>
<tr>
<td>Low**</td>
<td>113</td>
<td>19%</td>
<td>22</td>
</tr>
<tr>
<td>High-P†</td>
<td>122</td>
<td>34%</td>
<td>41</td>
</tr>
</tbody>
</table>

1-year eGFR<sub>MDRD</sub>

This analysis excluded allografts that failed before 2.5 years posttransplant

*Patients with high 1-year GFR (>40 mL/min) with increasing or stable eGFR<sub>MDMD</sub>.  
**Patients with low 1-year eGFR (<40 mL/min).  
†Patients with high 1-year eGFR (>40 mL/min) with strongly negative eGFR<sub>MDRD</sub> slope.  
eGFR, estimated glomerular filtration rate; NP, non-progressor; P, progressor.
CNIs seem to be the most effective drugs for inhibition of antigen triggering T cells.
Donor antigens in allograft trigger complex immune response involving T-cells, B-cells and complement

- **T cells**
  - **Regulatory T cells**
  - **Helper T cells**
  - **Effector T cells**

- **B cells**
  - **Plasmablasts**
  - **Plasma cells**
  - **Memory B cells**

- **Dendritic cells/macrophage**

**Donor antigen** (presented via MHC on donor-derived or recipient APC)

- **Activation** of the classic complement cascade with graft
- **Activation of intragraft APC and T cells**

**Complement**

Chronic low-level DSAs may have no detrimental effect, may have even protective effect\(^3\), some have been implicated in AMR\(^1\).

Development of DSA hypothesized to be related to inadequate immunosuppression and may appear any time after transplantation at varying levels\(^1\).

Increased risk of acute or chronic AMR\(^1\).

AMR, antibody-mediated rejection; DSA, donor-specific antibody; HLA, human leukocyte antigen.

DSAs are poorly characterized with low prevalence in renal transplant recipients

- The exact prevalence of donor-specific antibodies (DSAs) in kidney transplant patients is difficult to ascertain given that there is no standardized test for measuring DSA\(^1\).

- One study in 1043 kidney transplant recipients found \(~10\%\) of patients had DSA within 4 years of transplantation\(^2\).

- A separate study reported that among patients without detectable DSA at the time of transplantation, \(~11\%\) will have detectable DSA at 1 year and \(~20\%\) over the next 4 years.

- Antibodies may also be classified according to their activity against HLA\(^2,4\).
  - In a study of 1229 kidney transplant patients, 17\% had anti-HLA antibodies, one third of which had anti-HLA DSA\(^4\).

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DSA, donor-specific antibody; HLA, human leukocyte antigen; MMF, mycophenolate mofetil.
Antibody Mediated Deterioration of Allograft:

Donor-specific anti-HLA Antibodies after transplantation may be complement dependent or independent

## Consensus for clinical management of DSA

<table>
<thead>
<tr>
<th></th>
<th>screening*** de novo DSA</th>
<th>protocol biopsy</th>
<th>immunological risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSA-*</td>
<td>3-12 month</td>
<td><em>de novo</em> DSA+</td>
<td>low</td>
</tr>
<tr>
<td>DSA_actual_DSAHistorical+</td>
<td>&lt;1 month</td>
<td><em>de novo</em> DSA+</td>
<td>intermediate</td>
</tr>
<tr>
<td>DSA+**</td>
<td>&lt;3 month</td>
<td>&lt;3 month</td>
<td>high/very high</td>
</tr>
</tbody>
</table>

*in case of PRA0% (CDC) und DSA- allocation XM not mandatory. Cave: actual XM- is still mandatory
**even in case of desensitization therapy
***at least 1x / in given period

Tait BD et al, TP 2013, 95, 19-47
Cyclosporine vs. everolimus

Single center experience from the ZEUS and HERAKLES study

Σ 126 patients
[median 1059 d]

65 CSA [median 991 d]
- 7 DSAs (10.8%)
- 2 AMR
- 0 graft loss

61 EVR [median 551 d]
- 14 DSAs (23.0%)
- 8 AMR
- 4 graft loss

Incidence of *de novo* DSAs in KTx patients following conversion to an Everolimus-based CNI-free regimen

Kamar et al., Clin.Transplant 2013: 27: 455-462

**Results:**

No difference in incidence of DSAs or anti-HLA antibodies between both groups.

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**Table 1. Patients’ characteristics and immunological parameters**

<table>
<thead>
<tr>
<th></th>
<th>Everolimus group (n = 61)</th>
<th>Control group (n = 61)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HLA antibodies at baseline</td>
<td>14.75%</td>
<td>6.5%</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-HLA A/B antibodies at baseline</td>
<td>11.5%</td>
<td>5%</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-HLA DR/DQ antibodies at baseline</td>
<td>5%</td>
<td>6.5%</td>
<td>NS</td>
</tr>
<tr>
<td>DSA at baseline</td>
<td>0%</td>
<td>0%</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-HLA antibodies at last FU</td>
<td>20%</td>
<td>16%</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-HLA A/B at last FU</td>
<td>18%</td>
<td>10%</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-HLA DR/DQ at last FU</td>
<td>16%</td>
<td>10%</td>
<td>NS</td>
</tr>
<tr>
<td>De novo anti-HLA antibodies at last FU</td>
<td>16%</td>
<td>6.5%</td>
<td>NS</td>
</tr>
<tr>
<td>De novo DSA at last FU</td>
<td>9.8%</td>
<td>5%</td>
<td>NS</td>
</tr>
<tr>
<td>Time from baseline to DSA formation (months)</td>
<td>9.5 (6-19)</td>
<td>13 (6-25)</td>
<td>-</td>
</tr>
<tr>
<td>AMR</td>
<td>6.5%</td>
<td>0%</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Fig. 1. Incidence of donor-specific antibodies in both everolimus and calcineurin inhibitors groups.*

Figure and Tab modified from Kamar et al., Clin.Transplant 2013 27:455-462
Scheme of drug weaning and de novo DSAs

Hoshino J, Transplantation 2012; 93:1173-78
Translating in to clinical praxis
Renal function slope and DSAs

Jahre post Op

1/Kreatinin

CsA/MPS/P
Cert/MPS/P
A2309 post-hoc study: Safety events that met the criteria for further analysis

Lowest rates of AEs observed when EVR=3–8 ng/mL and CsA<100 ng/mL

*Increased as CsA exposure increased across most everolimus ranges
**No clear correlation with everolimus levels

AE, adverse event; CsA, cyclosporine A; EVR, everolimus; NODM, new onset diabetes mellitus; T, testosterone

Inhibition of mTOR and the podocyte: Hypothetical mechanisms

Hypothetical model: mTOR complexed to Rictor forms mTORC2, which phosphorylates Akt at Ser473

ALB, albumin; HMW, high molecular weight; LMW, low molecular weight; mTOR, mammalian target of rapamycin; VEGF, vascular endothelial growth factor; WT, wild type.

Case study

RA RAD, everolimus; MPS, mycophenolate sodium; P, prednisone; TAC, tacrolimus.
Over the last decade many trials investigated the potential of mTOR inhibitors to replace CNIs at different time points post-transplant.

De novo conversion to mTORi and CNI withdrawal or minimization

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Length of published FU</th>
<th>Time of conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Early Conversion:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMART¹</td>
<td>141</td>
<td>3 years</td>
<td>10–24 days</td>
</tr>
<tr>
<td>CENTRAL²</td>
<td>202</td>
<td>1 year</td>
<td>7 weeks</td>
</tr>
<tr>
<td>CONCEPT³⁴</td>
<td>192</td>
<td>4 years</td>
<td>3 Mo</td>
</tr>
<tr>
<td>Spare The Nephron⁵</td>
<td>299</td>
<td>2 years</td>
<td>30–180 days (mean 3.8 Mo)</td>
</tr>
<tr>
<td>A2309¹⁰</td>
<td>822</td>
<td>2 years</td>
<td>de novo</td>
</tr>
<tr>
<td>Herakles</td>
<td>499</td>
<td>2 year data now available</td>
<td>3 Mo</td>
</tr>
<tr>
<td>ZEUS⁶⁹</td>
<td>300</td>
<td>5 year data now available</td>
<td>4.5 Mo</td>
</tr>
<tr>
<td><strong>Late Conversion:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONVERT⁷</td>
<td>830</td>
<td>2 years</td>
<td>6–120 Mo (mean 3.3 years)</td>
</tr>
<tr>
<td>ASCERTAIN⁸</td>
<td>394</td>
<td>2 years</td>
<td>&gt;6 Mo (mean 5.6 years)</td>
</tr>
</tbody>
</table>

Multiple clinical trials have examined the different regimens for using everolimus in kidney transplantation.

- Improved renal function
- Risk of acute rejection
- Tolerability issues
- Practical challenges

1998

- Improved renal function
- Less neoplasia
- Improved renal function
- Better on severe BPAR
- CMV + BKV benefits (when used *de novo*)

2013

- CNI elimination*
- Late conversion
- CNI minimization

*everolimus is not approved for CNI elimination in kidney transplantation

BPAR, biopsy proven acute rejection; BKV, BK virus; CNI, calcineurin inhibitor; CMV, cytomegalovirus.
Immunosuppressive drug development in organ transplantation


AZA  CsA  TAC  MMF  SRL  MPA  EVR
STEROIDS

OKT3  ATG  Basiliximab Daclizumab  Campath  Rituximab  Belatacept

Eculizumab  Bortezomib

Next decade will probably focus on refinement of known drug treatments

ATG, anti-thymocyte globulin; ALG, Anti-lymphocyte globulin; AZA, azathioprine; CsA, cyclosporine; CsA-ME, cyclosporine microemulsion; EVR, everolimus; MMF, mycophenolate mofetil; TAC, tacrolimus; MPA, mycophenolic acid, SRL, sirolimus.
Long-term renal allograft survival has not improved

Decreased donor kidney transplant attrition rates in the US (n=164,480)

MMF, mycophenolate mofetil; Pred, prednisone; TAC, tacrolimus.

Poor renal function after transplantation has been shown to have multiple consequences.

- **CAN / IFTA**: Chronic allograft nephropathy; Interstitial fibrosis and tubular atrophy.
- **Graft failure**
- **Increased risk of mortality**
- **Increased CVD risk**
- **Increased health care costs**

Can, chronic allograft nephropathy; CVD, cardiovascular disease; IFTA, interstitial fibrosis and tubular atrophy.

mTOR inhibitors (everolimus) are central in several pathways relevant to post-transplant management

<table>
<thead>
<tr>
<th>Graft failure</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effectively prevents rejection when used in combination with reduced-dose CNIs</td>
<td>Tumor cell proliferation</td>
</tr>
<tr>
<td></td>
<td>Angiogenesis</td>
</tr>
<tr>
<td></td>
<td>Viral replication</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CVD</th>
<th>CAN/IFTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac hypertrophy</td>
<td>Endothelial cell proliferation</td>
</tr>
<tr>
<td>Homocysteine</td>
<td></td>
</tr>
<tr>
<td>Oxidative stress</td>
<td></td>
</tr>
</tbody>
</table>

CVD, cardiovascular disease; HIF-1α, hypoxia inducible factor 1 alpha; mTOR, mammalian target of rapamycin; mTORC, mammalian target of rapamycin complex; NFκB, nuclear factor kappa B; SREBP1, sterol regulator element binding protein 1.

**De novo** everolimus with low CNI provides protection against acute rejection in kidney transplantation

Efficacy at 12 months after kidney transplant


---

**BPAR (%)**

<table>
<thead>
<tr>
<th></th>
<th>A2309&lt;sup&gt;1&lt;/sup&gt;</th>
<th>A2309&lt;sup&gt;1&lt;/sup&gt;</th>
<th>ASSET&lt;sup&gt;2&lt;/sup&gt;</th>
<th>ASSET&lt;sup&gt;2&lt;/sup&gt;</th>
<th>US09&lt;sup&gt;3&lt;/sup&gt;</th>
<th>US09&lt;sup&gt;3&lt;/sup&gt;</th>
<th>SYMPHONY&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSA&lt;sub&gt;e&lt;/sub&gt; + EVR 0.75</td>
<td>CSA&lt;sub&gt;e&lt;/sub&gt; + MPA</td>
<td>TAC&lt;sub&gt;r&lt;/sub&gt; + EVR 1.5</td>
<td>TAC&lt;sub&gt;r&lt;/sub&gt; + EVR 1.5</td>
<td>TAC&lt;sub&gt;r&lt;/sub&gt; + EVR 0.75</td>
<td>TAC&lt;sub&gt;r&lt;/sub&gt; + EVR 0.75</td>
<td>TAC&lt;sub&gt;r&lt;/sub&gt;</td>
</tr>
<tr>
<td>CSA</td>
<td>100-200 ng/mL M0-M2</td>
<td>100-200 ng/mL M0-M2</td>
<td>4-7 ng/mL M0-M12</td>
<td>4-7 ng/mL M0-M12</td>
<td>4-7 ng/mL M0-M3</td>
<td>4-7 ng/mL M0-M3</td>
<td>3-7 ng/mL M0-M12</td>
</tr>
<tr>
<td>N</td>
<td>277</td>
<td>277</td>
<td>117</td>
<td>117</td>
<td>49</td>
<td>43</td>
<td>401</td>
</tr>
</tbody>
</table>

+=Results at 6-m

Everolimus is not approved for use in combination with tacrolimus in kidney transplantation.

CsA, cyclosporine; eGFR, estimated glomerular filtration rate; EVR, everolimus; MPA, mycophenolate acid; TAC, tacrolimus

De novo everolimus with CNI minimization provides renal benefits in kidney transplantation

Efficacy at 12 months after kidney transplant
Tedesco-Silva. Transplant Res. and Risk Manag., 2011

+Results at 6-m
Everolimus is not approved for use in combination with tacrolimus in kidney transplantation.
CsA, cyclosporine; eGFR, estimated glomerular filtration rate; EVR, everolimus; MPA, mycophenolate acid; TAC, tacrolimus
To meet the increasing need for kidney transplants, the criteria for the use of deceased kidneys has been expanded\(^1\).

Created a category of donors termed ‘expanded criteria donors (ECD)’\(^1\):

- Allografts are at risk for diminished post-transplant function\(^1\).

**ECD** = All donors aged over 60, and donors aged 50–59 plus ≥ two additional risk factors (cerebrovascular accident as a cause of death, history of hypertension, and serum creatinine >1.5 mg/dl prior to transplantation).

ECD, extended criteria donor.

CNI minimization is associated with reduced overall graft failure and death-censored graft failure

Graft survival with CNI minimization (17 trials, n=4131)

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>CNI sparing</th>
<th>CNI</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Events</td>
</tr>
<tr>
<td>Andres 2008</td>
<td>7</td>
<td>36</td>
<td>4</td>
</tr>
<tr>
<td>Budde 2010</td>
<td>0</td>
<td>66</td>
<td>0</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>2110</td>
<td>2021</td>
<td>100%</td>
</tr>
</tbody>
</table>

CNI minimization, compared with standard exposure CNI, was associated with:
- reduced overall graft failure (OR 0.73, \(P=0.009\))
- reduced death-censored graft failure (OR 0.73, \(P=0.03\))
- no difference in graft failure secondary to rejection (OR 0.67 [95% CI, 0.34–1.31], \(P=0.24\))

CAN, CVD and malignancy are the leading causes of graft failure and death with a functioning graft.
CI, confidence interval; CsA, cyclosporine A; eGFR, estimated glomerular filtration rate; EVR, everolimus; sCsA, standard CsA; tBPAR, treated biopsy-proven acute rejection
Novartis data on file
EVR: a multifaceted drug with the potential to improve long-term outcomes

CNI, calcineurin inhibitor; CMV, cytomegalovirus. EVR, everolimus.

CNI-Minimierung: Das Modell der Zukunft!

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Despite advances in acute rejection and 1-year graft survival over the past decade, long-term graft survival in kidney transplantation remains poor.

Declining posttransplant renal function is predictive of poor graft survival, patient mortality and is associated with:
- Increased risk of CV disease and mortality
- Increased healthcare cost

EVR has the potential to improve long-term outcomes compared with standard CNI regimens.

EVR plus low-dose CNI has been shown to be:
- Effective in preventing acute rejections in recipients of kidney transplantation
- Associated with improved renal function at Month 12 and 24

TRANSFORM is a superiority trial comparing EVR 1.5 mg plus low dose CNI with MPA plus standard dose CNI, which utilizes a novel and innovative primary endpoint comparing tBPAR and eGFR
- Designed to assess the clinical balance between rejection and renal function (graft outcome)
Renal transplantation: pre-CNI era

The first successful kidney transplant was performed between identical twins to avoid the immune-response related graft/patient survival\(^1\)

Minimal success has been observed with the use of total body irradiation for immunosuppression.\(^2,3\)

Disadvantages:
1. Cannot be quickly reversed
2. Does not have a long-lasting impact

CNI, calcineurin inhibitor.
1. Watson CJE and Dark JH. *British J Anaesthesia* 2012;108:i29–i42;
Considerable improvements have been made in acute rejection and short-term graft survival but…

ATG, anti-thymocyte globulin; CsA, cyclosporine; EVR, everolimus; MMF, mycophenolate mofetil.

43
\[\text{Primary deceased donor recipient} \]
\[\text{5-year survival 1991–2006}\]

Recipient survival, %

Year of transplant


\[84\%\] \[89\%\]

... long-term renal allograft survival has not improved...

Deceased donor kidney transplant attrition rates (n=164,480)

<table>
<thead>
<tr>
<th>Year of transplant</th>
<th>Years to overall graft survival half-life(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>6.8</td>
</tr>
<tr>
<td>1990</td>
<td>6.9</td>
</tr>
<tr>
<td>1991</td>
<td>7.3</td>
</tr>
<tr>
<td>1992</td>
<td>7.1</td>
</tr>
<tr>
<td>1993</td>
<td>7.4</td>
</tr>
<tr>
<td>1994</td>
<td>7.4</td>
</tr>
<tr>
<td>1995</td>
<td>8.0</td>
</tr>
<tr>
<td>1996</td>
<td>8.0</td>
</tr>
<tr>
<td>1997</td>
<td>8.3</td>
</tr>
<tr>
<td>1998</td>
<td>8.5</td>
</tr>
<tr>
<td>1999</td>
<td>8.6</td>
</tr>
<tr>
<td>2000</td>
<td>8.4</td>
</tr>
</tbody>
</table>


CAN is the leading cause of graft failure. CVD and malignancy are the leading causes of death in patients with a functioning graft.

Causes of death with a functioning graft:
- Cardiac and vascular: 37%
- Malignancies: 31%
- Infections: 18%
- Social: 6%
- Miscellaneous: 8%

Causes of graft failure:
- Acute rejection: 5%
- CAN: 68%
- Hyperacute rejection: 1%
- Vascular problems: 6%
- Technical problems: 2%
- Glomerulonephritis: 7%
- Non-compliance: 3%
- Other: 8%

Data from 2006–2010; n=1029 patients in Australia; n=772 patients in Australia.
CAN, chronic allograft nephropathy; CVD, cardiovascular disease.
ANZDATA registry 2011 report.
CVD, malignancy and infection are the major cause of death in patients with a functioning graft

### Causes of death with a functioning graft

<table>
<thead>
<tr>
<th>Cause</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac and vascular</td>
<td>37%</td>
</tr>
<tr>
<td>Malignancies</td>
<td>31%</td>
</tr>
<tr>
<td>Infections</td>
<td>18%</td>
</tr>
<tr>
<td>Social</td>
<td>6%</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>8%</td>
</tr>
</tbody>
</table>

*Data from 2006–2010; n=772 patients in Australia
CVD, cardiovascular disease.
Clayton P *et al.* ANZDATA registry 2011 report.
Transplant patients with diabetes and high cholesterol have a higher risk of associated IHD

Relative risk of IHD in renal transplant patients compared with FHS database

<table>
<thead>
<tr>
<th>Health Factor</th>
<th>Male Transplant Population (n=634)</th>
<th>Male FHS Population (n=2491)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus (Y/N)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>200–239</td>
<td>Male transplant population</td>
</tr>
<tr>
<td>35–44</td>
<td>240–279</td>
<td>Male FHS population</td>
</tr>
<tr>
<td>≥60</td>
<td>&gt;280</td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45–49</td>
<td>40–49</td>
<td></td>
</tr>
<tr>
<td>50–59</td>
<td>40–49</td>
<td></td>
</tr>
<tr>
<td>≥60</td>
<td>40–49</td>
<td></td>
</tr>
<tr>
<td>Cigarette use (Y/N)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FHS, Framingham Heart Study; HDL, high-density lipoprotein; IHD, ischemic heart disease.
Improvement in renal function after early conversion from a CNI- to an EVR-based immunosuppression regimen

An open-label, multicenter trial for de novo kidney transplant recipients: CENTRAL study

CNI, calcineurine inhibitor; CsA, cyclosporine; EVR, everolimus; ITT, intent to treat; mGFR, measured glomerular filtration rate.

EVR has the potential to improve key long-term outcomes for kidney transplant patients.

**Causes of graft failure**

- Acute rejection: 5%
- CAN (chronic allograft nephropathy): 68%
- Hyperacute rejection: 1%
- Vascular problems: 6%
- Technical problems: 2%
- Glomerulonephritis: 7%
- Non-compliance: 3%
- Other: 8%

**Causes of death with a functioning graft**

- Malignancies: 51%
- Infections: 18%
- Social: 6%
- Miscellaneous: 8%

EVR allows for CNI minimization

EVR reduces LVMi

EVR results in fewer malignancies vs. SoC

CAN, chronic allograft nephropathy; CNI, Calcineurin inhibitor; CVD, cardiovascular disease; EVR, everolimus; IFTA, interstitial fibrosis and tubular atrophy; LVMi, left ventricular mass index; SoC, standard of care

De novo everolimus with CNI minimization resulted in reduced incidence of CMV and BKV infections at Month 12 and 24.

CMV infection\(^1,2\)

<table>
<thead>
<tr>
<th>% of patients</th>
<th>EVR 1.5 mg</th>
<th>MPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-year</td>
<td>1.1%</td>
<td>8.4%</td>
</tr>
<tr>
<td>2-year</td>
<td>1.5%</td>
<td>6.2%</td>
</tr>
</tbody>
</table>

BKV infection\(^1,2\)

<table>
<thead>
<tr>
<th>% of patients</th>
<th>EVR 1.5 mg</th>
<th>MPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-year</td>
<td>0.7%</td>
<td>4%</td>
</tr>
<tr>
<td>2-year</td>
<td>0.7%</td>
<td>4.8%</td>
</tr>
</tbody>
</table>

BKV, BK virus; CMV, cytomegalovirus; EVR, everolimus; MPA, mycophenolate acid.
CMV infection is associated with reduced long-term survival in renal transplant patients

Prospective analysis of long-term survival of 471 kidney transplant recipients transplanted between 1994 and 1997

Probability of long-term patient survival

Low mortality risk, CMV–
Low mortality risk, CMV+
High mortality risk\(^a\), CMV–
High mortality risk\(^a\), CMV+

\(^a\)High-risk group includes patients with nephrosclerosis, diabetic nephropathy and amyloidosis.
CMV, cytomegalovirus.
CMV infection in renal transplant recipients is associated with impaired survival

Kaplan Meier analysis of overall recipient survival beyond 100 days posttransplantation

CMV infection during the first 100 days after kidney transplantation is associated with overall long-term mortality both in patients with a high or low risk of mortality

*Low risk of mortality: All other patients.
†High risk of mortality: Transplanted patients with a kidney diagnosis of nephrosclerosis, diabetic nephropathy and amyloidosis.
CMV, cytomegalovirus.
The risk of cancer is 3-fold higher in kidney transplant recipients than in general population.

Cancer risk\(^a\) in kidney transplant recipients versus the general population

\[^a\]Non-melanoma skin cancer, *in situ* lesions and lip cancer not included

ANZDATA, Australia and New Zealand Dialysis and Transplant Registry data; CI, confidence interval; RTx, renal transplant; SRR, standard rate ratio

Webster AC *et al.* Am J Transplant 2007;7:2140–51
Less CMV infections with everolimus than with MPA in KT: Pooled analysis of RCTs

EVR, everolimus; MPA, mycophenolic acid; CMV, cytomegalovirus.

**De novo** everolimus with CNI minimization resulted in fewer neoplasms

Incidence of neoplasms (AE)\(^1,2\)

<table>
<thead>
<tr>
<th></th>
<th>1 year</th>
<th>2 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVR 1.5 mg</td>
<td>3.3%</td>
<td>3.3%</td>
</tr>
<tr>
<td>EVR 3.0 mg</td>
<td>2.9%</td>
<td>2.5%</td>
</tr>
<tr>
<td>MPA</td>
<td>5.9%</td>
<td>4.8%</td>
</tr>
</tbody>
</table>

AE, adverse event; CNI, calcineurin inhibitor; EVR, everolimus; KTx, kidney transplant; MPA, mycophenolate acid.

De novo EVR: TRANSFORM study design
Simple trial design and clinically relevant

Group 1: CNI minimization (n=1,020)
Low CNI + EVR (C0 3–8 ng/mL)

Group 2: Standard of care (n=1,020)
Standard CNI + MPA

Opportunity for local & regional sub-studies

*Induction with basiliximab or ATG
**Stratified randomization within 12 hours for CsA or tacrolimus and donor type (living donors, deceased standard criteria donors, and deceased expanded criteria donors).
ATG, antithymocyte globulin; CNI, calcineurin Inhibitor; CV, cardiovascular; D, day; EVR, everolimus; LTGF, long-term graft function; MPA, mycophenolate acid; M, month; RDN, randomization; SCR, screening; Tx, transplantation.
Pascual J, et al. Poster presented at: 16th Congress of the European Society of Organ Transplantation (ESOT); September 8–11, 2013; Vienna, Austria.
**An innovative and novel endpoint based on current recommendations and latest thinking**

**tBPAR or eGFR* < 50 mL/min/1.73 m² at M12**

**tBPAR**
- Typical primary composite endpoint in transplantation registration trials includes death, graft loss, lost of follow up and tBPAR
- tBPAR is the largest contributor in the composite endpoint
- tBPAR is the only component that provides information about acute rejection relevant to require treatment
- Acute rejection (tBPAR) at 1-y has been associated with graft outcomes

**eGFR**
- Best estimate of renal function beyond mGFR
- eGFR or renal function at 1-y post-transplant has been associated with graft survival, all-cause mortality and CV risk*
- eGFR<50 represents a moderate level of renal dysfunction expected to be sensitive to the effects of acute and chronic rejection and nephrotoxic effects of immunosuppressants
- eGFR<50 may represent a surrogate for long-term survival

*(eGFR by MDRD4 formula)
CV, cardiovascular; eGFR, estimated glomerular filtration rate; tBPAR, treated biopsy-proven acute rejection.
Two-step hypothesis for chronic AMR (1): A complement-dependent step

- The first step of the two-step hypothesis is largely similar to the hypothesis for the mechanism of acute AMR

**Figure adapted from Stegall MD, et al. Nat Rev Nephrol. 2012;8:670–678 and Colvin RB. J Am Soc Nephrol. 2007;18:1046–1056.**

AMR, antibody-mediated rejection; DSA, donor-specific antibody; Ig, immunoglobulin; HLA, human leukocyte antigen.
Two-step hypothesis for chronic AMR (2): A complement-independent step

- Progression to graft loss may be due to inflammatory cell infiltration and prothrombotic factor expression independent of continued complement activation.
Primarily complement-independent hypothesis for chronic AMR

1. Intermittent or low-level activation of DSA
2. Complement activation leading to formation of C3a without C4d deposition
3. Chemotaxis of C3a causes infiltration of inflammatory cells
4. Development of transplant glomerulopathy subsequently leading to graft loss

AMR, antibody-mediated rejection; DSA, donor-specific antibody; HLA, human leukocyte antigen; NK, natural killer.

Figure adapted from Stegall MD, et al. Nat Rev Nephrol. 2012;8:670–678.
Complement-independent hypothesis for chronic AMR

1. DSAs directly activate endothelial cells

2. Development of transplant glomerulopathy subsequently leading to graft loss

AMR, antibody-mediated rejection; DSA, donor-specific antibody; HLA, human leukocyte antigen; MAC, membrane attack complex.

Figure adapted from Stegall MD, et al. Nat Rev Nephrol. 2012;8:670–678.
Complement-independent pathway of antibody mediated rejection (AMR)

- DSA bind HLA- or other donor antigen on endothelial cell
- Monocytes adhere to endothelium and infiltrate tissue
- Activation of mTOR-pathway -> proteinsynthesis and proliferation of endothelial and smooth muscle cells of vessels
- ⇒ subclinic vascular remodelling = antibody mediated accelerated arteriosclerosis

Aus: “Blickpunkt Medizin, Klinik und Praxis; Donorspezifische Antikörper (DSA) bei Nierentransplantation”, Advisory Boards FFM/Berlin 2013; Thieme; Sonderpuplikation in der DMW, 2013
Cyclosporine vs. everolimus

Single center experience from the ZEUS and HERAKLES study

Σ 126 patients [median 1059 d]
65 CSA [median 991 d]
7 DSAs (10.8%)
61 RAD [median 551 d]
14 DSAs (23.0%)

# Cyclosporine vs. everolimus

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cyclosporine group (n = 66)</th>
<th>Everolimus group (n = 61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal disease (GN vs. other)</td>
<td>32 vs. 34</td>
<td>30 vs. 31</td>
</tr>
<tr>
<td>Patients on randomized treatment at 12 months</td>
<td>58/65 (89%)</td>
<td>54/60 (90%)</td>
</tr>
<tr>
<td>Patients on randomized treatment at end</td>
<td>42/66 (64%)</td>
<td>40/61 (66%)</td>
</tr>
<tr>
<td>Steroid-free</td>
<td>41/66 (62%)</td>
<td>36/61 (59%)</td>
</tr>
</tbody>
</table>

*Never skip CNIs in frequent relapsing glomerulonephritis!*

*The rate in our center was max. 50%!*

*We never skip steroids in CNI withdrawal patients!*

## Combination of EC-MPS and everolimus

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cyclosporine group (n = 66)</th>
<th>Everolimus group (n = 61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean MPS-dose (mg/day)</td>
<td>1232 ± 278</td>
<td>1210 ± 259</td>
</tr>
</tbody>
</table>

**Effect on everolimus PK**

- **CsA withdrawal**¹: 36% lower everolimus exposure
- **Tacrolimus withdrawal**²: 8% lower everolimus exposure

**Effect on EC-MPS PK**

- **69% increase in MPA exposure**
- **6% increase in MPA exposure only**

---

CNI-elimination regimens

- Early CNI elimination* offers a long-term perspective on preserved renal function, but
  - Is not suitable immediately post-transplantation
  - Requires adequate dosing/TDM of the remaining drug
  - Exclusion of subclinical inflammation requires protocol biopsies
  - There is a risk of additional acute rejection episodes

- CNI-elimination regimens can be difficult to implement in the clinic
  - Requiring additional safety and dose-finding visits
  - May introduce additional/new drug-related adverse events

- Ongoing trials (MECANO, ELEVATE) will provide additional insights into the benefit/risk equation of early CNI elimination

*Everolimus is not approved for CNI elimination in kidney transplantation
CNI, calcineurin inhibitor; TDM, therapeutic drug monitoring.
The challenge is to improve long-term outcomes


CTS, Collaborative Transplant Study; UNOS, United Network for Organ Sharing.
## Causes of chronic allograft injury and morphological correlation

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Typical morphological changes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nonimmune damage</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Interstitial fibrosis and tubular atrophy due to CNI nephrotoxicity</strong></td>
<td>Arteriolar hyalinosis with peripheral hyaline nodules and/or progressive increase in the absence of hypertension or diabetes. Tubular cellular damage with isometric vacuolization</td>
</tr>
<tr>
<td>Interstitial fibrosis and tubular atrophy due to arterial hypertension</td>
<td>Fibrointimal enlargement with elastica reduplication, usually with hyaline arteriolar changes</td>
</tr>
<tr>
<td>Chronic urinary tract obstruction</td>
<td>Marked tubular dilatation. Tamm–Horsfall protein casts with interstitial and/or lymphatic extravasation</td>
</tr>
<tr>
<td>Viral nephropathy (especially BK virus nephropathy)</td>
<td>Viral inclusions in histology and immunohistology and/or electronic microscopy, variable degrees of tubulointerstitial inflammation and chronic nephritis</td>
</tr>
<tr>
<td>Bacterial pyelonephritis</td>
<td>Intratubular and peritubular neutrophils, with follicular lymphoid formation</td>
</tr>
<tr>
<td><strong>Immune damage</strong></td>
<td></td>
</tr>
<tr>
<td>Antibody-mediated chronic rejection</td>
<td>C4d deposits in PTC, with combinations of PTC basal membrane multilayering, glomerular basal membrane duplication (transplant glomerulopathy) or fibrointimal arterial enlargement without internal elastica duplication Other findings: inflammatory mononuclear cells in PTC, glomerulitis, interstitial plasmocytic infiltrate</td>
</tr>
<tr>
<td>T-cell mediated chronic rejection</td>
<td>Arterial intimal fibrosis with mononuclear infiltration in fibrotic areas with neointima formation</td>
</tr>
</tbody>
</table>

CAN is characterized by a progressive decrease in GFR, generally associated with proteinuria and arterial hypertension.

Changes in SCr levels and proteinuria may not accurately represent the underlying renal damage. Deterioration of renal function over time, determined through slope analysis, is a more accurate indicator of CRAI.

CAN, chronic allograft nephropathy; CNI, calcineurin inhibitor; CRAI, chronic renal allograft injury; GFR, glomerular filtration rate; PTC, peritubular capillaries; SCr, serum creatinine.

Low eGFR one year after transplantation is predictive of graft failure in subsequent years

Graft survival trends from 1 year after transplantation for patients with different levels of eGFR by MDRD (n=896)*

Patients with eGFR <40 mL/min 1 year after transplant have significantly lower graft survival (P<0.001)

*US data.
eGFR, estimated glomerular filtration rate; MDRD, modified diet in renal disease.
Early renal function is a strong predictor of 5-year graft failure

Adjusted hazard ratio of 5-year all-cause graft failure by 1-year eGFR (mL/min/1.73 m²)

Kidney-only transplant recipients, aged >18 years.
eGFR, estimated glomerular filtration rate (using the Modification of Diet in Renal Disease equation).
1-year eGFR predicts early graft failure

Graft survival trends from 1 year after transplantation for patients with different levels of eGFR by MDRD (N=896)

Patients with eGFR <40 mL/min experienced significantly poorer graft survival

Incidence of \textit{de novo} DSAs in KTx patients following conversion to an Everolimus-based CNI-free regimen

Kamar et al., Clin.Transplant 2013: 27: 455-462

Conversion to an Everolimus-based CNI-free immunosuppressive regimen has been associated with significantly better renal function maintained for 5 years\(^1\) as well as better outcome on de novo malignancies and BKV viremia\(^2\).

However, it is still under debate whether Everolimus and CNI have different impact on DSA development.

Kamar et al\(^3\) performed a \textit{retrospective case-control} study to compare the incidence of de novo DSAs in patients after conversion to an Everolimus-based, CNI-free immunosuppressive regimen (n=61) with (age, gender, induction therapy, date of Tx-) matched control group on standard CNI therapy (n=61).

\textbf{Methods:}

\begin{itemize}
  \item Stepwise conversion to Everolimus, median time after Tx= 22 (0.5-224) months
  \item Conversion for reasons of: IFTA or CNI-nephrotoxicity (n=26), participation in a clinical trial (n=26), BK-virus nephropathy (n=3)
  \item Biopsie proven that no features of cellular or humoral rejection had occurred
  \item HLA-antibody screening (Luminex) negative before conversion
\end{itemize}

Results:

No difference in incidence of DSAs or anti-HLA antibodies between both groups. Only the rate of increase in DSA within the everolimus group from 0% at baseline to 9.8% at last follow-up was significant but not within control group with 5% at last follow-up. However, no difference of any parameter was found between both groups.

Table 2. Outcome of kidney function and immunological parameters in the everolimus and control groups

<table>
<thead>
<tr>
<th></th>
<th>Everolimus group (n = 61)</th>
<th>Control group (n = 61)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Last follow-up</td>
</tr>
<tr>
<td>CsA/Tac/velpatascept</td>
<td>41/19/1</td>
<td>–</td>
</tr>
<tr>
<td>Anti-metabolite</td>
<td>87%</td>
<td>93%</td>
</tr>
<tr>
<td>Steroids</td>
<td>97%</td>
<td>75%</td>
</tr>
<tr>
<td>Creatinine level (μM)</td>
<td>136 ± 37</td>
<td>141 ± 54</td>
</tr>
<tr>
<td>aMDRD GFR (mL/min)</td>
<td>54 ± 18</td>
<td>56 ± 22</td>
</tr>
<tr>
<td>Anti-HLA</td>
<td>14.75%</td>
<td>26%</td>
</tr>
<tr>
<td>Anti-HLA A/B antibodies</td>
<td>11.5%</td>
<td>18%</td>
</tr>
<tr>
<td>Anti-HLA DR/DQ antibodies</td>
<td>5%</td>
<td>16%</td>
</tr>
<tr>
<td>DSA</td>
<td>0%</td>
<td>9.8%</td>
</tr>
</tbody>
</table>

CsA, cyclosporine A; Tac, tacrolimus; aMDRD, abbreviated Modification of Diet in Renal Disease; GFR, glomerular filtration rate; HLA, human leukocyte antibodies; DSA, donor-specific antibodies; NS, not significant.

Tab from Kamar et al., Clin.Transplant 2013: 27: 455-462
Results:

No difference in incidence of DSAs or anti-HLA antibodies between both groups. Only the rate of increase in DSA within the everolimus group from 0% at baseline to 9.8% at last follow-up was significant but not within control group with 5% at last follow-up. However, no difference of any parameter was found between both groups.

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<th>Table 2. Outcome of kidney function and immunological parameters in the everolimus and control groups</th>
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<tbody>
<tr>
<td><strong>Everolimus group (n = 61)</strong></td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td>CsA/Tac/belatacept</td>
</tr>
<tr>
<td>Anti-metabolite</td>
</tr>
<tr>
<td>Steroids</td>
</tr>
<tr>
<td>Creatinine level (μM)</td>
</tr>
<tr>
<td>aMDRD GFR (ml/min)</td>
</tr>
<tr>
<td>Anti-HLA</td>
</tr>
<tr>
<td>Anti-HLA A/B antibodies</td>
</tr>
<tr>
<td>Anti-HLA DR/DQ antibodies</td>
</tr>
<tr>
<td>DSA</td>
</tr>
</tbody>
</table>

CsA, cyclosporine A; Tac, tacrolimus; aMDRD, abbreviated Modification of Diet in Renal Disease; GFR, glomerular filtration rate; HLA, human leukocyte antibodies; DSA, donor-specific antibodies; NS, not significant.
Conclusion from this retrospective case-control study:

- Both groups showed incidence of de novo DSAs at last follow-up (FU)
- The incidence of DSAs was similar between both groups at last FU
- The increased incidence of DSAs between baseline and last FU was significant within the everolimus group (from 0% to 9.8%) but not within control group (0% to 5%). No significant inter-group difference.
- The incidence of AMR tended to be higher in the everolimus group.
- Low number of events makes interpretation difficult
- Multivariate modeling for factors associated with DSA occurrence did not identify everolimus regimen as risk factor for DSAs.
- Only younger recipient age was identified as factor associated with de novo DSA development.

=> Younger recipient age is well known risk factor for non-compliance.
CAN, CVD and malignancy are the leading causes of graft failure and death with a functioning graft

**ANZDATA registry data 2007–2011**

**Causes of kidney graft failure**

- CAN, 69%
- Glomerulonephritis, 6%
- Vascular, 5%
- Acute rejection, 5%
- Noncompliance, 4%
- Technical problems, 1%
- Other, 9%

**Causes of death with functioning graft**

- Malignancy, 31%
- CVD, 34%
- Infection, 19%
- Miscellaneous, 10%
- Social, 7%

**a**Data from 2007–2011; n=1071 patients in Australia; **b**Data from 2007–2011; n=852 patients in Australia.

CAN, chronic allograft nephropathy; CVD, cardiovascular disease; IFTA, interstitial fibrosis and tubular atrophy.

Striking a balance between EVR exposure, tBPAR, and eGFR

*Time normalized

CsA, cyclosporine; eGFR, estimated glomerular filtration rate; EVR, everolimus; tBPAR, treated biopsy-proven acute rejection.

EVR plus reduced CsA is associated with LVH regression in renal transplant recipients

Left ventricular hypertrophy 1 year after kidney transplantation is associated with reduced long-term survival and increased risk of de novo heart failure.

LVMi was significantly lower in the EVR plus low CsA group than in controls.

*Randomized controlled single-center trial in kidney transplant patients.
CsA, cyclosporine; EVR, everolimus; LVH, left ventricular hypertrophy.
The risk of cancer is 3-fold higher in kidney transplant recipients than in general population

Cancer risk in kidney transplant recipients versus the general population

- **Female**: 95% CI SRR 3.2 (2.94–3.45)
- **Male**: 95% CI SRR 2.6 (2.40–2.78)

ANZDATA (1980–2002) n=12,633; RTx, ≥1 cancer 10.5%

- Non-melanoma skin cancer, *in situ* lesions and lip cancer not included.

CI, confidence interval; RTx, renal transplant; SRR, standard rate ratio.

In the presence of mTOR inhibitors, the TORC1 dependant stages of viral replication are blocked.

Recent studies conclude that while CMV protein synthesis is reliant on mTOR kinase during early infection, CMV replication appears to be mTOR kinase independent in later stages.

Chronic viral (opportunistic) infection associated malignancies have the most increased incidence

EBV, Epstein-Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HHV8, human herpesvirus 8; HPV, human papilloma virus.

Practical challenges associated with CNI elimination regimens

- Treatment regimen change in stable transplant recipients
- Additional hospital visits
- Period with more frequent monitoring for acute rejection required
- Risk of introducing additional/new adverse events

CNI, calcineurin inhibitor.