mTOR inhibitors lights and shadows
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Disclosure of Interest
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Scientific advice to companies:
Amgen, Astellas, Pfizer, Novartis, Roche, BMS

The details of each Disclosure of Interest are available at the Invited Speakers' desk (located in the Registration Area).

Slide 2
Good afternoon to everyone and let me first thank the organising and scientific committee for the kind invitation to talk here with you about mTOR inhibitors.
Caravaggio is the real master of light and shadows. So, since I cannot even think to compete with him, I will try to address this issue, our issue with a more systematic approach.

Slide 5

SWOT Analysis

Strengths
Weaknesses
Opportunities
Threats

Let us try to do a SWOT analysis looking at the strength, the weaknesses, the opportunities and the threats of this class of drugs.
Before doing that, I will try to underline that we are dealing with a really complicated system. The target of rapamycin is included in two complexes within the cells: mTORC1 that is sensitive to rapamycin and mTORC2 on which the action of rapamycin is still largely unclear.

They respond to stimuli like growth factors, energy status, amino acids and hypoxia.
and they control survival, translation, autophagy, cytoskeleton organization. We are really talking about the key switch in cell metabolism. So, all the beneficial effects that we would see could be really great and all the side effects that we're going to see could be really depressing.

Slide 9

SWOT Analysis

Strengths
Weaknesses
Opportunities
Threats
Let's start with the strengths. When we talk about the strengths of this class of drugs, of course,

Slide 10

**Conversion to sirolimus causes an improvement in renal function**

![Graph showing GFR over time with SRL Conversion and CNI Continuation](#)

*Values adjusted for baseline by ANCOVA.

Schena FP et al. Transplantation, 2008

the first thing that comes to mind is the possibility to reduce the CNI load and preserve renal function, graft function. This was demonstrated with this first, big late conversion trial, the CONVERT trial. As you can see the renal function of the patients converted to Sirolimus is always significantly better than the patients continuing on CNI.

Slide 11

**Efficacy on Renal Function of Early Conversion from Cyclosporine to Sirolimus 3 Months After Renal Transplantation: Concept Study**

<table>
<thead>
<tr>
<th></th>
<th>SRL group (n = 85)</th>
<th>CsA group (n = 96)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr CI C-G formula (mL/min)</td>
<td>68.9 [65.9;71.8]</td>
<td>64.4 [61.7;67.1]</td>
<td>0.017</td>
</tr>
<tr>
<td>eGFR simplified MDRD formula (mL/min)</td>
<td>61.2 [58.2;64.1]</td>
<td>53.9 [51.2;56.7]</td>
<td>0.002</td>
</tr>
<tr>
<td>Creatininemia μM/L</td>
<td>117.4 [110.7;124.2]</td>
<td>132.3 [126.1;138.5]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>SRL group (n = 59)</th>
<th>CsA group (n = 75)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured GFR using iohexol (mL/min)</td>
<td>67.3 [63.7;71.0]</td>
<td>60.3 [57.0;63.5]</td>
<td>0.004</td>
</tr>
</tbody>
</table>


This was confirmed also in an early conversion trial, the CONCEPT trial. Again, the Sirolimus patients presented a renal function always better than the ones continuing on CNI.

Slide 12
Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: an open-label, randomised, controlled trial

But not all the trials work in the same way and give us the same information. Indeed, in the ORION study we could not see any difference in renal function, in graft function.
In any case, besides the graft function, graft morphology is for sure preserved by this therapy. This was an initial observation by Flechner in de novo kidney transplantation but also we demonstrated that when we considered patients continuing on CNI for 2 years, all the patients progressed in their lesion, whereas the patients converted to Sirolimus preserved their morphology.
In these patients, we observe a clear reduction in the progression of tubulointerstitial fibrosis and also in the glomerulosclerosis.

Rapamycin reduces the progression of interstitial fibrosis and glomerulosclerosis in patients with chronic allograft dysfunction.

Rapamycin reduces PAI-1 glomerular and tubulointerstitial expression in patients with chronic allograft dysfunction.

and these went together with a significant reduction in profibrotic mediators like PAI-1.
But nowadays our main problem is not graft survival itself but death with a functioning graft. When we talk about death with a functioning graft, we talk about cardiovascular disease, malignancy and infection.

**Causes of death with a functioning graft**

But nowadays our main problem is not graft survival itself but death with a functioning graft. When we talk about death with a functioning graft, we talk about cardiovascular disease, malignancy and infection.

**Slide 19**

**Rapamycin inhibits primary and metastatic tumor growth reducing neoangiogenesis**

These are 3 key strengths of these drugs; indeed Guba and collaborators already demonstrated that Rapamycin may inhibit primary and metastatic tumour growth through its anti-angiogenic effects.

**Slide 20**
A few years later, always the Guba groups, demonstrated that Rapamycin also demonstrates an inhibition of lymphangiogenesis that is clearly implicated in metastasis.

Slide 21

Inhibition of the mammalian target of rapamycin impedes lymphangiogenesis

S Huber et al Kidney Int 2007

Something that we don't think of very often about is that Rapamycin can induce also cell differentiation like here in melanoma cells.
and we demonstrated that Kaposi sarcoma indeed, expresses high levels of protein ID2 and this regulates cell differentiation and when we treat this patient with Rapamycin, this expression is significantly reduced.

Stallone G et al. Am J Transplant 2010

Kaposi sarcoma skin lesion before and one month after conversion to sirolimus

In the clinical setting, we learned that converting patients from CNI to Sirolimus could change significantly the natural history of Kaposi sarcoma but what about other malignancies?

Slide 24

**Actual incidence of any de novo malignancies by drug regimen**

Kaufmann et al, Transplantation, 2005

We have a couple of registry observations. Here Kaufmann from the UNOS demonstrated that patients on mTOR inhibitors presented significantly fewer malignancies. All malignancies, and, above all, non-skin malignancies.

Slide 25

**Immunosuppressive therapy**

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Kaposi</th>
<th>NHL</th>
<th>Solid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IRR (IC 95%)</td>
<td>IRR (IC 95%)</td>
<td>IRR (IC 95%)</td>
<td>IRR (IC 95%)</td>
</tr>
<tr>
<td>CNI</td>
<td>No</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.1 (0.6-2.0)</td>
<td>1.6 (0.4-6.4)</td>
<td>1.0 (0.1-7.3)</td>
</tr>
<tr>
<td>mTORi</td>
<td>No</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0.5 (0.4-0.7)</td>
<td>0.5 (0.2-0.9)</td>
<td>0.3 (0.1-1.1)</td>
</tr>
<tr>
<td>Induction</td>
<td>No</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.1 (0.9-1.4)</td>
<td>1.4 (0.9-2.3)</td>
<td>0.9 (0.4-1.7)</td>
</tr>
</tbody>
</table>

We confirmed recently this data with the Italian Registry. As you can see all malignancies and PTLD as doctor Grinyo showed before but also solid malignancy, the incidence of all of them was significantly reduced in patients on mTOR inhibitors.

Slide 26
As for interventional trials, the CONVERT study, doctor Green has already shown this data, demonstrated that, although the incidence of malignancy was not a primary outcome, patients converted to Sirolimus presented a lower incidence of neoplastic disease.

Very recently, another intervention trial demonstrated that patients with previous skin cancer randomised to Sirolimus basically did not present recurrence of their skin malignancy.
In heart transplant, our colleagues clearly demonstrated that treatment with mTOR inhibitors reduces the left ventricular mass
and this was confirmed by a small pilot elegant study by Ernesto Paoletti also in kidney transplant recipients.

**Everolimus prevents immune response-related intimal thickening**

*Mouse carotid artery allografts (5 weeks p.o.)*

Besides the effect on Left ventricular mass, mTOR inhibitors can significantly reduce intimal hyperplasia directly and this was also demonstrated.
in humans in the study presented by Eisen in the New England with IUS. Basically they demonstrated that graft vasculopathy was significantly reduced by treatment with Everolimus.

**Slide 33**

**Everolimus reduces the incidence of viral infection in de novo heart transplants**

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>1.5 mg of Everolimus (N=209)</th>
<th>3.0 mg of Everolimus (N=211)</th>
<th>Azathioprine (N=214)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>151 (72.2)</td>
<td>162 (76.8)</td>
<td>150 (70.1)</td>
</tr>
<tr>
<td>Bacterial</td>
<td>69 (33.0)</td>
<td>80 (37.9)</td>
<td>53 (24.8)</td>
</tr>
<tr>
<td>Wound</td>
<td>14 (6.7)</td>
<td>11 (5.2)</td>
<td>7 (3.3)</td>
</tr>
<tr>
<td>Fungal</td>
<td>16 (7.7)</td>
<td>24 (11.4)</td>
<td>19 (8.9)</td>
</tr>
<tr>
<td>Aspergilus</td>
<td>4 (1.9)</td>
<td>5 (2.4)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Candidiasis</td>
<td>10 (4.8)</td>
<td>18 (8.5)</td>
<td>16 (7.5)</td>
</tr>
<tr>
<td>Viral (cytomegalovirus, herpes simplex, herpes zoster)</td>
<td>31 (14.8)</td>
<td>36 (17.1)</td>
<td>67 (31.3)</td>
</tr>
</tbody>
</table>


Finally, and directly linked also with malignancies, infections in particular, viral infections are significantly reduced by this drug.

**Slide 34**
Everolimus reduces the incidence of Cytomegalovirus infection in de novo renal transplants


This is a review of the 3 major studies with Everolimus demonstrating that this drug was able to reduce the incidence of CMV infection both in the whole population and in patients with prophylaxis.

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Direct and indirect effect of mTOR inhibition on viral replication
This is due basically to an effect, a direct effect on the cell machinery that promotes viral replication and on the CD8 T cells that are devoted to the antiviral response.

Slide 36

**SWOT Analysis**

**Strengths**

**Weaknesses**

**Opportunities**

**Threats**

The weaknesses.

Slide 37

**Comparison of Sirolimus Plus Tacrolimus Versus Sirolimus Plus Cyclosporine in High-Risk Renal Allograft Recipients: Results From an Open-Label, Randomized Trial**

A. Osama Galber, Barry D. Kahn, Charles Van Buren, Seth L. Schulman, Joseph Scarola, John F. Nейlan, and for the Sirolimus High-Risk Study Group

This drug has one major weakness and I think that all of us have experienced this in our everyday clinical practice. This is a randomly chosen trial. As you can see, in this trial we got a 40% dropout rate. Basically, drug discontinuation is something that we experienced in the trial but also in everyday clinical life.

Slide 38
• Lymphocele and wound healing
• Delayed graft function
• Hyperdyslipidemia
• Mouth ulcers
• Anemia
• Edema
• Pneumonitis

This was due basically to the side effects of this drug: lymphocele and wound healing, delayed graft function, hyperdyslipidemia, mouth ulcers, anaemia, oedema, pneumonitis.

Slide 39

Conversion From Calcineurin Inhibitors to Sirolimus Maintenance Therapy in Renal Allograft Recipients: 24-Month Efficacy and Safety Results From the CONVERT Trial

Francesco P. Schena,1,2 Michael D. Pascoe,3 Josefina Albera,4 María del Carmen Bial,5 Rainer Oberbauer,6 Daniel C. Brennan,6 Josep M. Comistol,1 Lorraine Racusen,7 Martin S. Polinsky,8 Robert Goldberg-Alberts,9 Hualiu Li,10 Joseph Scarcella,10 and John F. Nylam9 for the Sirolimus CONVERT Trial Study Group10

Transplantation 2009;87: 233–242

Most of them are dose-related. Indeed in the CONVERT study in the first 6 months after therapy, the period when you use the highest drug levels, you observe a significant increase in adverse events. Whereas in the next 6 months their incidence is significantly reduced.

Slide 40
Now let me change to SWOT analysis. I don't like SWOT analysis because it closes with threats. I don't like to close with threats. I'm optimistic, I prefer to close with good news. So let's get rid of the threats before. We have two major threats for these drugs.

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**Pattern of proteinuria following conversion from CNI to mTOR inhibitor**

One is proteinuria. We experienced that converting from CNI to mTOR inhibitors we saw the increase in proteinuria. We thought that maybe this is due to CNI withdrawal.

Slide 42
But the same thing happened also when we converted from Azathioprine to mTOR inhibitors.

**Slide 43**

**High Sirolimus Levels May Induce Focal Segmental Glomerulosclerosis De Novo**

Emmanuel Letavernier,* Patrick Bruneval,† Chantal Mandet,‡ Jean-Paul Duong Van Huyen,§ Marie-Noëlle Péraldi,‖ Imed Helal,* Laure-Hélène Noël,§ and Christophe Legendre*  


Indeed, we learned that there was a direct nephrotoxic effect of the drugs. High doses of the drug can induce changes in the glomerulus that are similar to focal segmental glomerulosclerosis.

**Slide 44**
We demonstrated that, indeed, patients exposed to high levels of the drug presented a significant reduction in the main proteins linked to the slit diaphragm within the podocytes, nephrin, podocine, CD2AP.

The drug levels again, play a key role. Something that we have to keep in mind anyway is that in experimental models, in several proteinuric experimental models, mTOR inhibitors were able to ameliorate, to improve tubulointerstitial damage linked to proteinuria.
This was true in a model of membranous nephropathy and also in a model of diabetic nephropathy.

Since we are talking about diabetic nephropathy, here comes the second threat: diabetes, post-transplant diabetes is significantly higher when we add to CNI an mTOR inhibitor.
Insulin sensitivity in rapamycin-converted Kidney Transplant recipients

A few years ago we demonstrated that this effect was basically due to a reduced insulin sensitivity. Indeed, patients converted from CSA or tacrolimus, all of these patients were taking also prednisone, decreased their insulin sensitivity after conversion to Sirolimus.

Teutonico et al JASN 2005

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Insulin resistance and rapamycin-induced mTORC2 activation
Recently, we used a less elegant but more practical way to measure insulin sensitivity, the HOMA index and again the patients treated with Rapamycin presented a high resistance. Interestingly we could associate this resistance, the increased resistance to insulin to an increased peripheral activation of mTORC2 as demonstrated by phosphorylation of serine 473 of AKT.

Now I don't want to try to sell you that diabetes is good but here I'm showing this slide on one of the best known anti-ageing proteins Klotho but I can assure you that now we understand that whatever can make you survive longer, can extend your lifespan will also decrease your insulin sensitivity.

So, this is something that we have to keep in mind when we go on and talk about opportunities. I see with this drug at least two big opportunities that we still need to work on.
The first one is tolerance. We know that Tregs are key cells in tolerance induction, in transplant tolerance induction and we also know very well that mTOR inhibitors can preserve Tregs in our patients since they don't touch IL-2 signalling and IL-2 effects.

But what's really interesting is that mTOR inhibitors can also induce de novo generation of alloantigen specific regulatory T cells from CD4 positive T cells. So, it's not only preservation but also induction of Tregs.
CD8⁺T cells and tolerance

Human T suppressor cells (TS) expressing the CD8⁺CD28⁻FOXO3⁺ phenotype, use a restrict repertoire of TCR V genes and recognize peptides presented by MHC class I molecules.

These CD8⁺CD28⁻ T cells suppress the activation of CD4⁺T cells inducing the downregulation of costimulatory molecules CD40, CD80, and CD86 and the upregulation of ILT3 and ILT4 in antigen-presenting cells.


There is something else that we need to consider, we saw that Rapamycin can influence CD8 function and there is a particular class of CD8⁺Cd28⁻ cells that have been suggested to play a role in tolerance. These cells according to Suchu-Foca group can modulate antigen presenting cells and make them tolerogenic through the upregulation of two proteins: ILT3 and ILT4.

Ig-like transcript (ILT) 3 and 4

ILT3 and ILT4, expressed by monocytes and DCs, belong to a family of Ig-like inhibitory receptors.

The subset of ILT receptors including ILT3 and ILT4 mediate inhibition of cell activation by recruiting a tyrosine phosphatase (SHP-1).

ILT3\textsuperscript{high}ILT4\textsuperscript{high} DC induce anergy in alloreactive CD4⁺CD45RO⁺CD25⁺ T cells converting them into Treg.

Colonna et al. Semin Immunol 12: 121, 2000

These are two inhibitory receptors that, when expressed in antigen presenting cells, can induce energy in alloreactive CD4 positive cells converting them into Tregs.
Indeed, in our experience, converting patients from CNI to mTOR inhibitors induces the expression of dendritic cells of both ILT3 and ILT4. Particularly on plasmacytoid dendritic cells that we learn play a key role in transplant tolerance.
Conversion to Sirolimus induces an increase in circulating Tregs and CD8⁺CD28⁻ T cells

This was strictly associated with an increase in the number of circulating Tregs and with an increase in the number of CD8 and CD28-T cells.

Deceased donor transplants from ECD & DCD donors

Stallone et al. under revision

USRDS 2010
The second opportunity: Senescence. Our donors are growing older and older

Slide 60

P16INK4a protein expression, a senescence marker, in biopsies from transplants with TA/IF compared to their implantation biopsy

and when we transplant these old kidneys, after transplantation we observe an accelerated senescence, cellular senescence. Now, we would need something to stop ageing.

Slide 61

Live Long and Prosper

and reduce significantly the ageing process.

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mTOR inhibition and aging
Indeed, this is taken from a very recent review in JCI, mTOR1 inhibition by Rapamycin, as well as dietary restriction, can induce autophagy and can reduce protein synthesis. These two events significantly reduce cellular stress and damage accumulation, thus prolonging the lifespan of our cells. This is something that maybe we can try to use to reduce the cellular senescence in our kidney transplants.

Slide 64

**Sirolimus and kidney transplantation**

- Oral Ulcers
- Edema
- NODAT
- proteinuria
- Hyperdyslipidemia
- Delayed wound healing
- Interstitial pneumonia (rare)

*So if we want to make a balance, to look at the balance, for sure we have weaknesses, we have heavy weaknesses for this drug, we have two important major threats but still if we put together the strengths of this drug and their opportunities I still believe that there should be some space in our immunosuppressive therapy to use mTOR inhibitors.*

Slide 65

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Now, let me thank all the people that work in this field and allowed me to present our data. Thank you very much.
Chairman: Thank you very much Dr. Grandaliano. Are there any questions or comments?

Question: There have been so many complications over the years suggesting these drugs are inferior to tacrolimus or calcineurin inhibitors on long-term renal transplant survival also they are more expensive than the calcineurin inhibitors especially Everolimus?

Prof. Grandaliano: I don't think that we should think of the use of these drugs versus calcineurin inhibitors, Rainer Oberbauer was thinking of a kind of sequential therapy. There are periods of our transplant life where we absolutely need calcineurin inhibitors and there are other periods where the calcineurin inhibitors can damage our transplant or our patient. So we have to try to put these things together, I mean not thinking one versus the other but maybe integrating the two immunosuppressive drugs.

Chairman: Sorry, another question over there, yes.

Question: Thank you sir for this nice presentation. I want to ask about the recommendation by the last KDIGO Clinical Practise Guidelines regarding avoidance of Sirolimus in patients with eGFR 40 ml/min and below. Is this still valid or has this theory been challenged?

Prof. Grandaliano: No, it's still valid. For sure I think that one of the things that caused some depression in people using these drugs in the past was that we thought that this drug could help us in restoring or preserving renal function and morphology also late when the damage was really late. I think that the late conversion is something that is not good for the transplant and for the patients. If we believe in conversion, we need to convert early and not wait until the renal function, the graft function is low.

Question: Thank you.

Chairman: Yes please.

Question: Well, thank you for that elegant SWOT analysis but actually there was a recent publication of a meta-analysis study of retrospective nature extracting data from US-RDS data on mortality and graft loss of mTOR2 two years and at 10 years after survival. Alone or – showed increased graft loss and mortality in the mTOR2 arm? How do you see this?

Prof. Grandaliano: Yes, I saw the study of course. There are several biases of course given the retrospective nature of the study. Indeed, when we saw the study, we went back to our Italian registry and we tried to do the same analysis. I didn't show it because it was a preliminary observation but we observed exactly the contrary. I mean the presence of mTOR inhibitors significantly reduced mortality. But it's really preliminary and we were considering 8.000 patients and 1.800 with mTOR inhibitors.

Question: What happens in the course of Sirolimus when you're using Sirolimus and GFR drops to 30 ml/min, what is your approach do you convert back to some other agent stopping Sirolimus?

Prof. Grandaliano: Maybe before doing something I would go and look at what's happening in
the graft with the biopsy and I would like to go back and look at the level that I kept of Sirolimus before doing anything else. But first of all, I wouldn't go on and look at the biopsy.

Question: I suppose the biopsy shows ATN sub-acute rejection, some fibrosis, so what approach do you have?

Prof. Grandaliano: If the immune-mediated damage is prevalent of course, I'll go back to tacrolimus, if there is something else...

Question: ATN.

Prof. Grandaliano: I would just reduce the dose.

Question: --fibrosis – thanks.

Chairman: Are there any questions? If there aren't any questions I want to thank all the speakers and the audience and I close the session.