Thank you very much Francesco, Maryvonne and I would like to thank the organisers for this kind invitation to participate in this session on diagnosis in transplantation.

I’m going to try to address how we can personalize much more than we have done so far in transplant medicine, using perhaps biomarkers.
Many of the things I will talk about have been previously alluded to by Ondrej. Just to remind you that nowadays we have seen that we have been able to decrease the incidence of acute rejection from the 90s to the 2000s to less than 15% but this has not resulted in a reduction of graft loss at least in renal transplantation and this is true for diseased donors and living donor transplants. So perhaps this is because the classical injuries in renal transplantation per se suggest nephrotoxicity and nowadays you know that graft attrition is in fashion because of humoral rejection and also we can’t forget chronic T cell-mediated rejection.

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This results in a sharp improvement, in the reduction of graft attrition during the first year after transplantation but with no impact on long-term graft attrition which results in a very modest impact on predicted half-life of the graft.

Slide 5

Nowadays you know that we have a lot of patients targeting probably the most essential steps in allograft reaction.

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But the classical or the gold standard therapy that has been used in many, many transplant injuries in the so-called SYMPHONY study which is in fact a quadruple therapy providing us rejection rates between 15-20% and much higher when we use low dose sirolimus.

More recently, you have seen even using this quadruple therapy in a controlled regimen as suggested in the BENEFIT study in the context of the belatacept development programme probably we have been very, very surprised because of the low range of acute rejection even using this classical therapy. But with the new agents we have seen more conventional rejection rates mainly due to T cell-mediated rejections and very few of them were antibody-mediated rejections.

Not Personalized Medicine in Renal Transplantation

- Gold standards (quadruple therapies)
- Low rates of early acute rejection
- Persistent metabolic, cardiovascular and neoplastic morbidities.
- Low comorbidity regimens?
- Trial and error approach
- Failures additionally derive in conservative approaches
- Risk of late graft failure

So nowadays we think that we have to address a low toxicity regimen but we don’t really know how we can do that because as I told you, the majority of teams use classical quadruple therapies aiming to have initial low rates of acute rejections. But we have to see our patients dyeing because of cardiovascular events and even neoplasia in the long-term. So low comorbidity regimens are very different in different centres and at times we try to extremely reduce or even eliminate toxic immunosuppression when using the trial and error approach and the failures resulting from these strategies additionally derive in more conservative approaches because of the tremendous concern or late graft failure.
So, nowadays you know that we have exactly the same causes we have seen for 2-3 decades explaining or accounting for late graft losses: CNI nephrotoxicity is under debate, some clinicians think that they are not so harmful but they are there and at the end the risk is that we’re not using a very classical concept which is an elementary lesion called interstitial fibrosis tubular atrophy which means not too much derived from probably toxic and immune-mediated injuries.

You know that in renal transplantation we usually use an important load of immunosuppression early after the surgery in order to prevent this acute rejection. Then in a second phase we try to reduce the doses of immunosuppression in order to avoid these long-term comorbidities but with the concern of increasing the risk of late antibody-mediated rejection. We have not seen real benefits in reducing cardiovascular and neoplastic morbidities.
In renal transplantation in fact, we are in some way very rude if I can say that or very primitive in comparison with other medical specialities let’s say diabetes, hypertension and pneumonia where the targets are clearly related to the pharmacodynamic effects of the drugs used. For instance, in the control of diabetes we monitor glucose and not insulin levels. Or in the blood pressure we monitor daily blood pressure not the levels of hypertensive agents. But in the case of transplantation the only monitoring we use as a routine is the pharmacokinetics of immunosuppressives given to these patients.

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Current evaluation of Transplant risk → management of IMS

Current surrogate endpoints used in the clinic:

- Conventional markers of graft dysfunction (serum creatinine, FGt, liver function tests...)
- Pharmacokinetics of IMS to indirectly evidence the immune status
- Pathological changes derived from allograft biopsies → “Photofinish”

→ Fail to anticipate the initiation of allograft dysfunction → little predictive information
→ Not always linked to long-term outcomes
→ Do not offer information on the recipient’s anti-donor and overall immune status

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So in the end we have to manage these patients very empirically and in addition we also use the classical metabolic parameters, let’s say serum creatinine, pharmacokinetics and at times very few teams also use protocol biopsies in order to get information about the preservation of the renal parenchyma. But this is very static information; this is like a photo finish saying in that particular time what is the real preservation of the structure --- in the long-term.
So it seems that we have to refine much more what we can do with our patients and because of that you know that biomarkers may help us especially in the midterm.
At the beginning we use biomarkers pre-transplant in order to prevent early immunological failures and in the midterm we also may use biomarkers in order to assess the immunological risk, the degree of allograft damage and at times we may also be able to use biomarkers in order to assess the state of immunosuppression or the induction of potential tolerance.

**Slide 13**

**Biomarkers in renal transplantation**

- > 4800 publications.
- Genomic, proteomic, metabolomic and tissular biomarkers.
- Diagnostic and prognostic value?.
- Useful tools to assess therapeutic maneuvers?
- Biomarkers of acute graft injury, chronic graft damage, immune response.
- Functional immunological biomarkers

If you review the literature of biomarkers in renal transplantation, you’ll see that there are a huge number of papers published over the past decades, many of them related to genomics, proteomics, metabolomics and also tissue biomarkers used for diagnostic and prognostic values.

Ondrej mentioned what has been done in these – in order to assess the biomarkers in tolerant patients. It’s less clear which may be the contribution of these tools to assess therapeutic manoeuvres-. You’ll see that in the literature you’ll have biomarkers for acute graft injury, chronic damage or immune response.
But I think that probably one of the interesting ways to integrate that would be to use functional biomarkers mainly related to T and B cell responses, gene signatures in the graft and in the periphery and also perhaps we’ll be able to assess the role of potential T cell subsets especially Tregs and the T effector memory cells and we have even done some work characterising the cell phenotypes in the graft parenchyma.

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But relating to the question of Francesco to Ondrej perhaps we might use an integrated approach on the combined use of these biomarkers using functional immunoassays I suggest ELISPOT, gene expression in the tissue and in the periphery, urinary proteomics and correlating all these findings with graft biopsy. Because these are quantifiable probably THEY may help us to predict the outcome of the graft. Not all of them but the majority of them are not very invasive except graft biopsy and can be conducted from the early periods after transplantation. But we need to conduct proper clinical trials in order to validate these biomarkers early after transplantation related to the use of induction of therapies or in maintenance re-establish patients in order to address specific minimization immunosuppressive strategies.

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Here you have an interesting work not in renal transplantation but in liver transplantation recently published by Sanchez-Fueyo using liver tissue expression and after the withdrawal of immunosuppression in almost 100 patients showing that they detected 40% of tolerant grafts. You know that empirically in liver transplantation approximately 20% of the recipients may survive with minimal amounts of immunosuppression or even without immunosuppression at all. They identified gene signatures correlating with this state of tolerance.

On the other hand and this French group from Nantes also identified 33 out of 49 genes in patients with operational tolerance which differentiated very much these patients from healthy subjects or patients with chronic rejection.
But perhaps in clinical transplantation it might be more relevant not only to detect those gene signatures or those biomarkers related to operational tolerance which is only true for an extremely minimal proportion of patients but probably the use of biomarkers could make sense in order to go far to extreme immunosuppression minimization in order to achieve a state of proper tolerance. Because of that perhaps functional biomarkers may help.

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If we keep in mind this cartoon concerning the adaptive alloimmune responses with all the actors participating in this response,

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you'll see that the pool of T cell effector and memory cells accounts for a lot because the balance of these effector cells with regulatory T cells may account for the immune response and rejection. On the other hand these T cells are highly specialized and elicit strong responses accounting for early and late acute rejection.

So assessing the role of these memory cells by using functional – and moreover these T cells are very resistant to regulation. It has been nicely shown in this recent paper in the mouse
but you know that in human beings we have a much more sophisticated immune system with an expanded pool of T effector memory cells and this suggests that the experiences observed in rodents may not really be translated to human beings and because of that we need to conduct additional work in the clinical setting and not just extrapolating the findings of these rodent models.

By using the functional biomarkers; IFN-γ ELISPOT, these authors identified that those patients having acute rejection had stronger responses than those free of acute rejection. Moreover, in post-transplantation the intensity of response in the ELISPOT correlated with renal function. The higher the response, the worse the renal function, the higher the serum creatinine.
We in our team have also been interested that was a work cooperating with Chairtè in Berlin that – the participation of direct and indirect pathways in renal transplantation.

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Presence of Direct and Indirectly alloreactive T cells in longstanding renal Transplant patients

-> 38 Kidney TX patients > 2 years (2-10)
-> All on CNI

Presence of anti-donor indirect and directly primed memory/effecter T cells in the long term

Bestard O et al. JASN 2008

and you may see here that after transplantation direct pathway participates in the immune response in a higher level than in the indirect pathway very specific for donor specific responses.

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Moreover, the direct responses correlated with renal function, the stronger the response the poorer the renal function or in other words the hypo responders had better renal function than dose responders and also patients having late acute rejection also had stronger responses and this was also correlated with the degree of HLA compatibility.

On the other hand, in the indirect pathway those patients having strong responses in the indirect pathway had proteinuria but in this study there was no graft histology. An interesting concept is that the indirect pathway takes the role in the long-term matter of transplantation. So it seems that indirect responses will replace direct responses observed at the early times after transplantation.
So on the other hand we have also studied these ELISPOT responses in a tolerogenic protocol free of calcineuric inhibitors and steroids and what we have seen is that by using this strategy we saw that almost 60% of patients became hyporesponders and that the response was specific for the donor antigens and not related to the third party which recorded strong responses.

Also we correlated the ELISPOT responses in this data of acute rejection high responders in rejectors versus non-rejectors and the hyporesponders had a better renal function.
I think that probably for the sake of time I will skip these slides. Just mentioning that on the other hand the intensity of the response correlated with the integrity of renal parenchyma. Those patients having low responses have a better preserved renal parenchyma.

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Prospective assessment of T-cell sensitization for IMS individualization?

Non-randomized, Pilot study for Selection of either CNI-based or CNI-free Immunosuppressive regimen depending on donor-specific T-cell alloreactivity (IFN-y ELISPOT) in De Novo Standard-risk renal allografts

- IFN-y ELispot at pre-Tx and at 6 month
- 5-Month allograft biopsy for histological assessment
- 6-Month DSA

And on the other hand, we also saw the presence of T regulatory cells infiltrating these allografts because of that we conducted a non-randomised study,

Slide 32
a prospective study individualising the immunosuppression depending on pre-transplant IFN-γ ELISPOT those hyporesponders were given CNI free regimens and those high responders were given CNI based drugs.

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6-M d-s IFN-γ Elispot change and Graft function evolution

So what we have seen in order to summarise our findings is that patients, regardless of the pre-transplant immune responses, had a close correlation between these post-transplant responses and renal function. Those negative patients had the better renal function in comparison with those who persisted as responders.

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We were also able to establish a nice correlation between the degree of response and the observation of subclinical rejection in protocol biopsies with a higher sensitivity and specificity.

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Finally, I would close this talk saying that because of these initial findings we have decided to conduct a prospective and randomised trial based on the use of biomarkers in this case IFN-γ ELISPOT and will establish two sets of patients: those in which we will give a particular immunosuppression depending on the pre-transplant status and in the other group we’ll give the classical immunosuppression minimization strategy versus a standard immunosuppression in order to know whether the use of biomarkers adds an additional benefit in order to induce donor specific hyporesponses reducing acute rejection and better preserving renal function.

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Finally, I would like to thank my contributors especially Oriol Bestard who did the major part of this work. Thank you very much.