**THERAPEUTIC DRUG MONITORING OF TACROLIMUS-PERSONALIZED THERAPY: SECOND CONSENSUS REPORT**

**Supplementary material; supplementary tables**

**Supplementary Table 1: Clinical studies investigating T-cell proliferation and activation after tacrolimus exposure.**

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| **Author**  **(ref)** | **Aim of the study** | **Main findings** |
| **Weimer1** | - compare the effect of Tac on humoral immune response in renal Tx patients | - switching IS from CsA to Tac suppresses costimulatory ligands and adhesion molecules (CD28, CD40L, CD54), Th1 responses and CD4 helper activity |
| **Härtel 2** | - investigate PD of Tac (e.g. CD25 and CD69) in renal Tx patients | - CD25 and CD69 expression was diminished in the presence of 25µg/L Tac |
| **Barten3** | - monitor the conversion of CsA to Tac in heart and lung Tx patients | - increased Tac concentrations did not influence T-cell proliferation, but inhibited CD25 expression |
| **Barten4** | - assess PD effects of Tac-based IS in heart Tx patients | - Tac inhibited expression of IL-2, TNF-a, IFN-g, PCNA and CD134 more than CsA, but expression of IL-4, CD25 and CD95 were comparable |
| **Bai5** | - explore the regulatory function of Tac on CD4/CD8 T-cell subgroups and co-stimulators in liver Tx patients | - expression of T- cell subgroups returned to normal level  - expression of CD28 and ICOS on T-cells decreased, while CD152 expression on T- cells increased  - higher regulatory effect of Tac on T cell subgroups compared to CsA |
| **Ashokkumar6** | - investigate CD154+ T-cells in liver Tx patients | - allospecific CD154+Th memory cells, but not CD154+ T cytotoxic memory cells were inhibited by increasing Tac concentrations |
| **Kim7** | - compare brand-name and generic Tac in liver Tx patients regarding CD56+ T- cells | - level of CD56+ T-cells were higher in brand-name than in generic TAC group |
| **Laskin8** | - measure T-cell proliferation in Tac-treated pediatric renal Tx patients using a CFSE assay | - 24.3% and 25.3% reduction of CD4+ and CD8+ T cell proliferation (Tac trough level: 7.4 ng/mL) |
| **Shi9** | - investigate whether the changes of Treg/CD4+ T-cell ratio are associated with allograft tolerance and survival in Tac-treated liver Tx patients | - CD3+CD4+ T-cells and CD4+/CD8+ T-cells were lower in Tac-treated patients than in controls  - percentage of Th17 cells in CD4+ - cells were higher in short- and mid-term Tac-treated patients  - percentage of NK cells were not different in Tac-treated patients compared to controls |

CD4/8/25/28/40L/54/56/69/154, cluster of differentiation 4/8/25/28/40L/54/56/69/154; CFSE, carboxyfluorescein succinimidyl ester; CsA, cyclosporin A; IS, immunosuppression; NK, natural killer; p-ERK, phosphorylated extracellular signal-regulated kinase; (p-)p38MAPK, (phosphorylated) phospho38-mitogen-activated protein kinase; PD pharmacodynamics; Tac, tacrolimus; Th (17), T helper (17); Treg, regulatory T-cell; Tx, transplantation

Supplementary Table 2. Overview of some of the data analysis approaches involved in previously reported pharmacodynamic studies for CNIs.

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| Authors (Reference) | Calcineurin inhibitor | Transplant type | Number of patients | Number/type of data analysed | Data analysis approach/estimation methods(\*) | Model evaluation methods |
| Millan et al 10 | CsA and Tac | Renal transplant | 16 out of 65 stable patients (CsA)  10 out of 65 stable patients (Tac) stable renal patients >7 and 5 years, respectively.    12 healthy individuals for basal measurements | 3 sampling times per patient (Cmin, C2h and Cmax) | Non parametric spearman correlation test between CAN at 0 and 2 h and Tac or CsA Cmin, C2h, Cmax and AUC values | - |
| Koefoed-Nielsen et al 11 | Tac | Renal transplant | 18 patients | 6 sampling times per patient  ( 0,1,2,3,4 and 6 h) on Day 14 post-transplantation/blood concentrations | Pearson’s correlations  between each Tac blood concentration and AUC of CNA inhibition | - |
| Koefoed-Nielsen et al 12 | Tac | Renal transplant | 29 patients (CsA)  19 patients (Tac) | 6 sampling times per patient  ( 0,1,2,3,4 and 6 h) on Day 14 post-transplantation/blood concentrations | Visual inspection and t-student based statistical comparisons | - |
| Koefoed-Nielsen et al 13 | Tac | Renal transplant | 23 stable patients (CsA)>7 years  17 stable patients (Tac) >4 years | 5 sampling times per patient  ( 0,1,2,3,4 and 4 h) on three occasions (Day 1 and 180 and 2h on Day 90) for a 6 month period/blood concentrations | Visual inspection and t-student based statistical comparisons | - |
| Fukudo M et al 14 | CsA  Tac | Living-donor liver transplant | 40 de novo liver transplant 30 patients (Tac) and 10 patients (CsA) | C0 (Tac) and C0 and C2h (CsA) for 14 days post-transplantation / Tac, CsA blood concentrations and CAN phosphatase activity in PBMC | Direct inhibitory Emax model. Non-linear- mixed-effects models implemented in NONMEM® | - |
| Blanchet et al 15 | Tac | Liver-transplant | 14 patients during the first three months post-transplantation | 14 patients (0, 2.3.4. 6 and 9h post dosing on days 8, 21 and 90 post-transplantation | Hill model between CAN AUCeff0-12h and TAC AUC0-12 values | Nor Internal neither external model evaluations were reported |
| Abdi ZD et al16 | CsA, Tac and MPA | Renal transplant | 222 patients , treated with CsA (126 out 222) or Tac (96 out 222) and MPA given to all of them | Association between MPA AUC, CsA C2h and Tac C0h and acute rejection, graft loss and death    Association between MPA AUC, CsA C2h and Tac C0h and CMV infection or disease | Time-to-event models  Parametric survival modeling for Acute rejection graft loss or death  Logistic regression model for CMV infection  Maximum likelihood estimation method  NONMEM® | Non parametric bootstrap analysis  Evaluation of TTE models through Kaplan-Meier visual predictive plots  Logistic regression models evaluated by comparison of the distributions of proportions of CMV events of the observed and simulated datasets |
| Zheng S et al.17 | Tac |  | 24 healthy subjects | 18 Tac blood concentrations per subject for the 96 hours post-administration,  Urine concentrations at 8 different intervals over the 96 hours post-administration.  SNPs in the CYP3A5 gene were determined | A Semi-physiological model was developed to evaluate the effect of CYP3A5 polymorphism on intrarenal metabolism and tubulo-epithelial exposure to Tac/The model was implemented in SAAM II® | Comparison of observed and predicted amounts of tacrolimus excreted unchanged in urine |

(-) Nor internal neither external model evaluation was performed

(\*) data provided as long as it was explicitly given in the publication

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