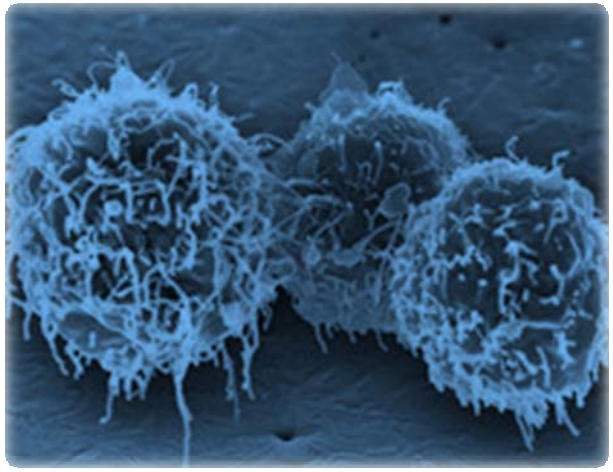
"Introducing the Next Generation of Cell Mediated Immune Monitoring!"

Novel Epigenetic Immune Cell Markers Enable Standardized Immune Monitoring from Frozen Whole Blood or Tissue during Clinical Trials



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Disclosure

Thomas-Oliver Kleen, PhD
is an employee of
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Challenges to the Feasibility of CMI Analysis

- Conventional methods for monitoring functional, cell based immune biomarkers (FACS-ICS, ELISpot) require living and functional cell material - sample management a core challenge
- Assay specific stability of blood samples and specimens is limited in particular when involving sample collection and shipping from multiple clinical sites (Shipping & Freeze/Thaw cycles)
- Obtaining sufficient quantity and quality of blood or biopsy material by diverse clinical sites is putting strain on IRBs and patient compliance
- Costly training and resources for sample preparation at clinical sites (PBMC isolation, cryopreservation, shipping)

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Benefits of Epigenetic CMI Analysis

- Discovery of cell type specific epigenetic markers allows precise and robust quantitation of immune cells in all human samples
- Requires only small amounts of sample (0.1 to 1ml whole blood) permitting add on of CMI monitoring for most clinical studies
- Standardized tests are based on quantitative PCR targeting genomic DNA, making readout stable and allowing samples on site to be simply frozen and easily shipped
- Enables monitoring of patients in large multicenter studies, retrospective studies, routine monitoring and objective comparison of results between different studies

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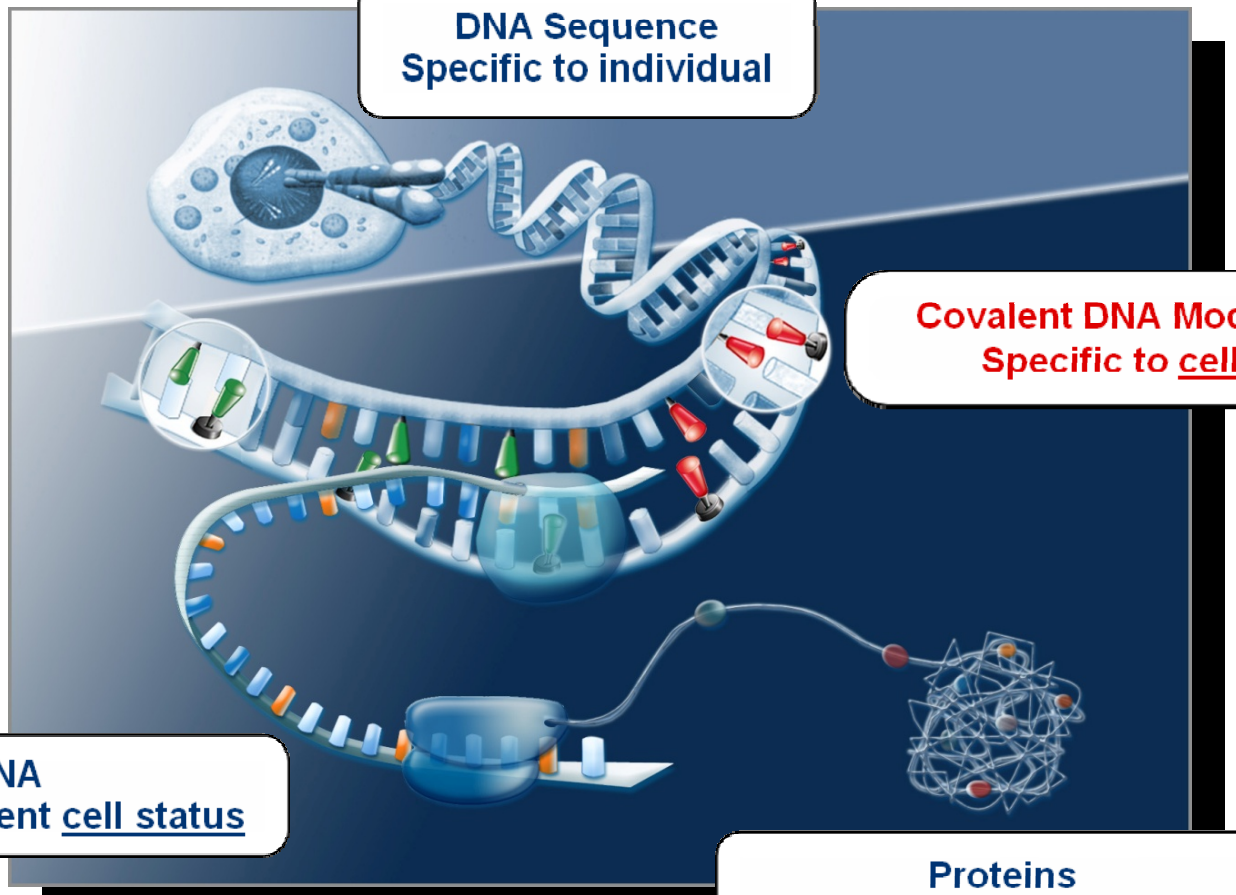
Epigenetic Modification of DNA Specific for the Cell Type



DNA Sequence
Specific to individual

Covalent DNA Modification
Specific to cell type

mRNA
Specific to current cell status

Proteins
Specific to current cell state

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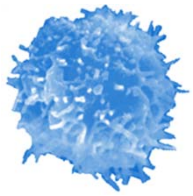
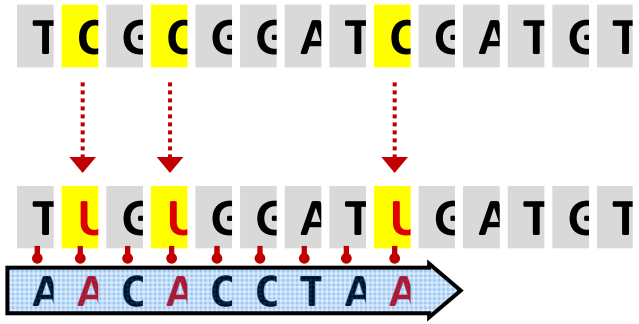
Cell Type Specific Epigenetic qPCR-Assays

If Example Target cell type is:

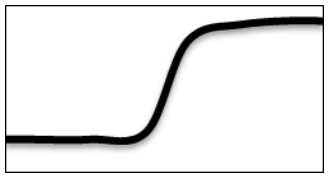
- Regulatory T-cell (Treg)



- Treg Marker sequence CpGs are **de-methylated**



qPCR Product :



Process

Blood or Tissue

↓

Cells

↓

Native DNA

↓

Bi-Sulfite conversion

↓

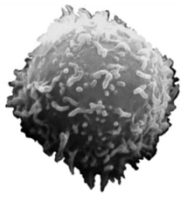
PCR primer

↓

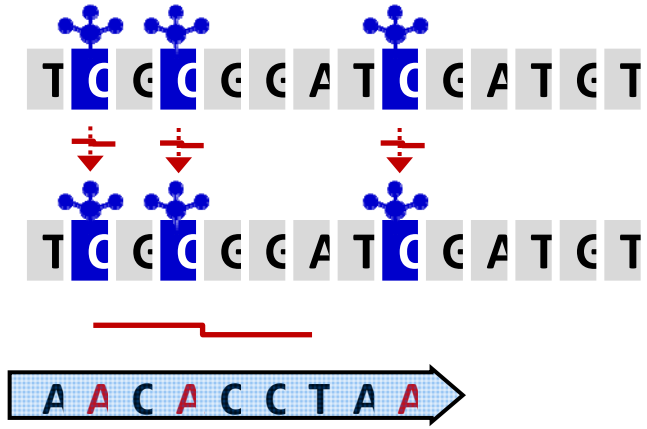
qPCR reaction

Then Non-target cell types are:

- All non-Treg cells e.g. activated T-, B- CT-, NK-Lymphocytes and Leukocytes



- Treg Marker sequence CpGs are **methylated**



No qPCR Product :

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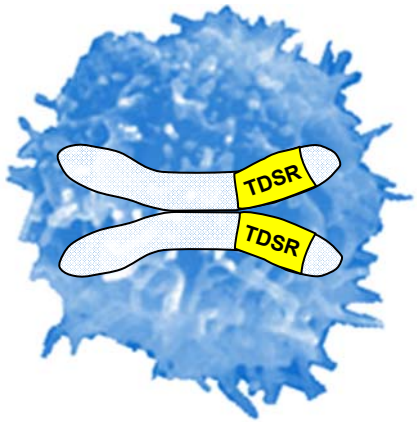
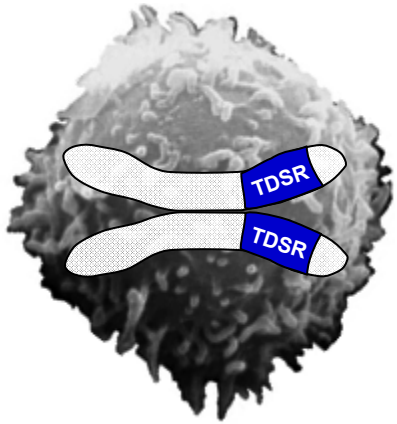
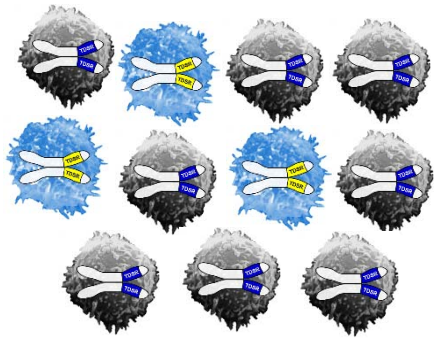
Epigenetic qPCR Cell Counting Principle

T-reg Cell	Any Other Cell Type	Blood Sample
		
2 demethylated copies detected by PCR	**NO demethylated copies detected by PCR**	**30% T-reg Cells are detected by PCR**

- **Detection of epigenetically active (de-methylated) gene copies by quantitative PCR allows robust and precise cell counting**
- **Parallel measurements of epigenetic reference systems e.g. housekeeping gene GAPDH or specific plasmid standards allow for total cell number determination**

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Epigenetic Marker Discovery – Differential Methylation Hybridization

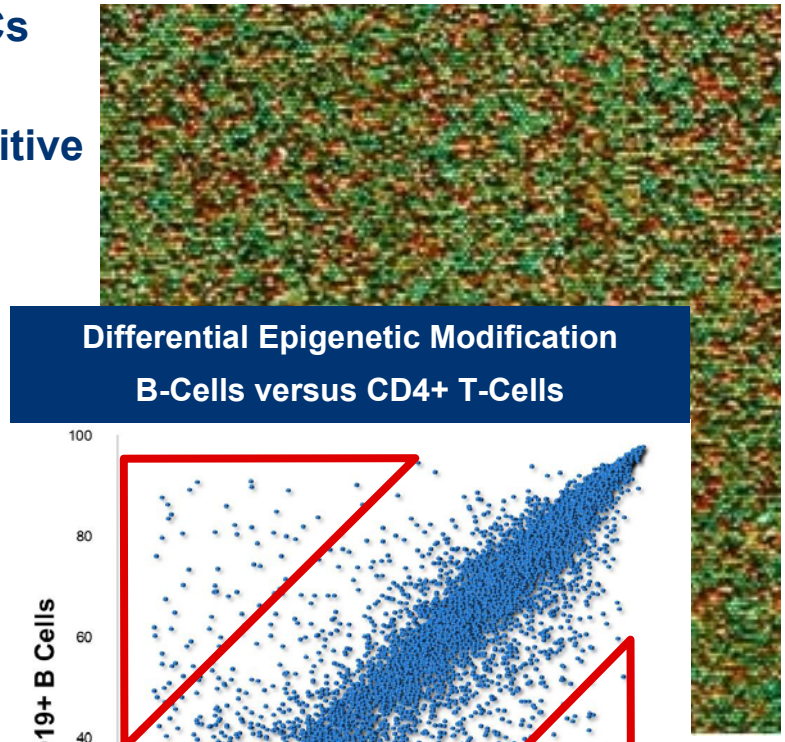
Process

- Genomic DNA from specific cell type (e.g. FACs sorted) is digested and linkers are ligated
- Fragments are digested with methylation sensitive restrictases
- PCR amplification of unrestricted fragments
- Unspecific fragmentation, labeling
- Detection on custom Affymetrix chip
- Differential Comparison with other cell types

Chip characteristics

- Nearly all human genes covered
- 50,000 fragments
- Multiple features per fragment

Alternative: Literature based candidate genes



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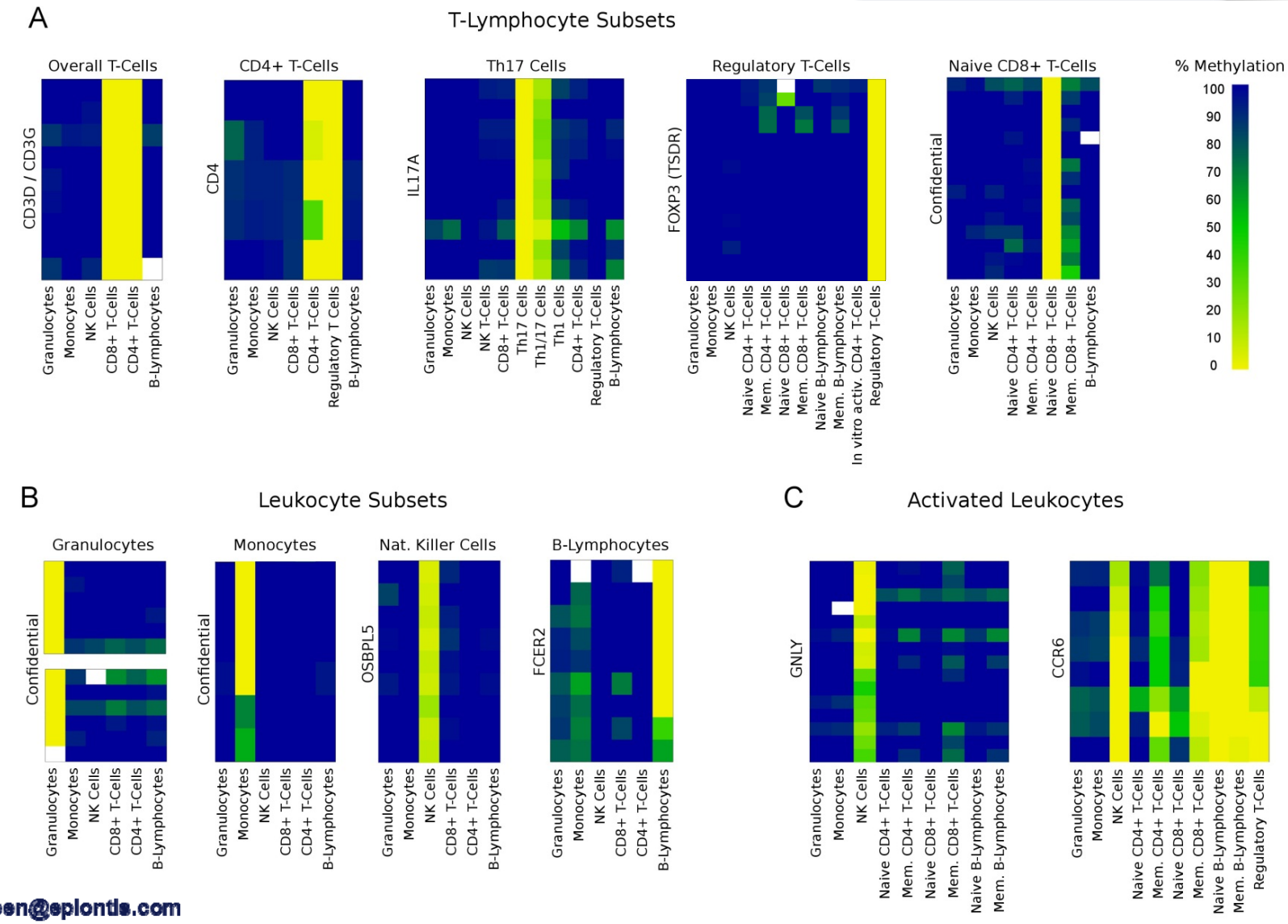
Unique, IP-protected Epigenetic Markers of Immune Cells



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Development of Relevant Assays

Established Assays

- Regulatory T cells
- TH17-cells*
- Overall T cells (CD3)
- B cells
- NK cells (CD56 dim)
- CCR6
- Granulysin
- Neutrophil Granulocytes
- Naive CD8 T cells
- CD4 T cells*
- Monocytes

(* Currently being validated)

In Earlier Development

- Granulocytes
  - Eosinophils
  - Basophils
- Naive CD4 T cells
- Memory CD4 T cells
- Memory CD8 T cells
- Naive B-cells
- Memory B-cells
- Central Memory T cells
- Myeloid Suppressor cells
- Dendritic Cells
- TH1/TH2-cells

In Advanced Development Stage

- CD8 T cells

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Sample Material Needed

Assay	*Validated* amount of DNA from whole blood *required* in *µg*	*Minimum* volume of whole blood *recommended** in *µl*	Minimum number cells from whole blood *recommended**
FOXP3	1.3	100	700,000
CD3	0.3	50	350,000
CCR6	1.2	100	700,000
GNLY	1.0	100	700,000
NK Cells	1.33	100	700,000
B Cells	0.38	50	350,000
Naive CD8 Cells	0.33	50	350,000
Granulocytes	0.2	50	350,000
Monocytes	0.15	50	350,000

*Assumes 7,000 (5,000-10,000) leukocytes per µl whole human blood

- Less than 1 ml whole blood allows to run the entire panel of cell types, twice
- Enabling clinical trials and immune monitoring in settings never before thought feasible

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Types of Samples that can be measured with Epigenetic Assays

Blood :

- EDTA
- Citrate
- Heparin
- Fresh/Frozen
- Whole Blood
- PBMC

Tissue Biopsies :

- Fresh/Frozen
- RNA keeper/RNA later
- FFPE/paraffin embedded

**less extensive validated than Blood*

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Clinical Trial Testing Expertise and Quality Management (QM), Quality Systems (QS)

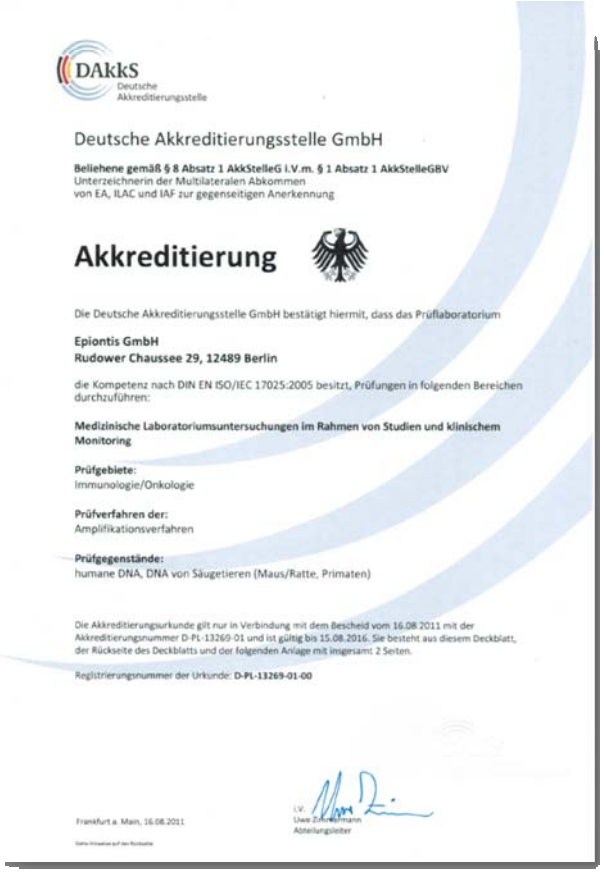
Epiontis‘ CRO Services Experience

(as of September 2012)

- Clinical Samples tested since 2008: 38,000
- Completed and running clinical trials: 21
- Pilot studies, academic collaborations: 270

QM and QS

- Accredited according to DIN EN ISO/IEC 17025:2005 by DAkkS
- In compliance with GLP and ICH guidelines
- Continued, successful on-site audits of QM and QS by pharmaceutical and biotech companies
- Regular reviews of QM and QS by Epiontis and clients



DAkkS
Deutsche Akkreditierungsstelle

Deutsche Akkreditierungsstelle GmbH

Beliehene gemäß § 8 Absatz 1 AkkStelleG i.V.m. § 1 Absatz 1 AkkStelleGBV
Unterzeichnerin der Multilateralen Abkommen
von EA, ILAC und IAF zur gegenseitigen Anerkennung

Akkreditierung

Die Deutsche Akkreditierungsstelle GmbH bestätigt hiermit, dass das Prüflaboratorium

Epiontis GmbH
Rudower Chaussee 29, 12489 Berlin

die Kompetenz nach DIN EN ISO/IEC 17025:2005 besitzt, Prüfungen in folgenden Bereichen durchzuführen:

Medizinische Laboratoriumsuntersuchungen im Rahmen von Studien und klinischem Monitoring

Prüfgebiete:
Immunologie/Onkologie

Prüfverfahren der:
Amplifikationsverfahren

Prüfgegenstände:
humane DNA, DNA von Säugetieren (Maus/Ratte, Primaten)

Die Akkreditierungsurkunde gilt nur in Verbindung mit dem Bescheid vom 16.08.2011 mit der Akkreditierungsnummer D-PL-13269-01 und ist gültig bis 15.08.2016. Sie besteht aus diesem Deckblatt, der Rückseite des Deckblatts und der folgenden Anlage mit insgesamt 2 Seiten.

Registrierungsnummer der Urkunde: D-PL-13269-01-00

Frankfurt a. Main, 16.08.2011

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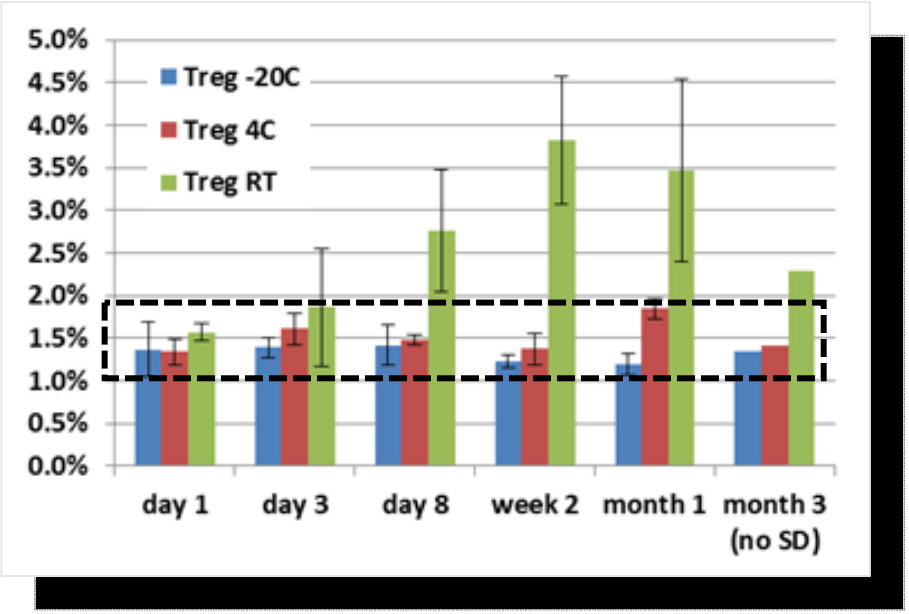
Marker Stability for Epigenetic Assays in Whole Blood Samples and Tissue

Validated minimum stability in example Treg

- 1 day at room temperature
- 1 week at 4°C
- > 1 year at -20°C
- > 1 year stability experiments are running



Measurment capabilities on various aged sample types (non-validated)

- > 4 years at -20°C: whole blood
- > 8.5 years at -20°C: fresh tissue
- > 11 years at RT or -20°C: FFPE

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Example Rationale for Epigenetic Treg Monitoring

- Increased Treg frequencies measured by mRNA or protein FOXP3 were found in various cancers – indicating possible role during tumor establishment and maintenance
- Inability of human FOXP3 mRNA or protein to differentiate between Treg and activated T-cells limits its usefulness as biomarker
- Technical demands of sample logistics and processing for current FOXP3 assays restricts its application as clinical marker
- Novel method of Treg-specific DNA de-methylation within the FOXP3 locus can reliably measure Treg in peripheral blood and solid tissues

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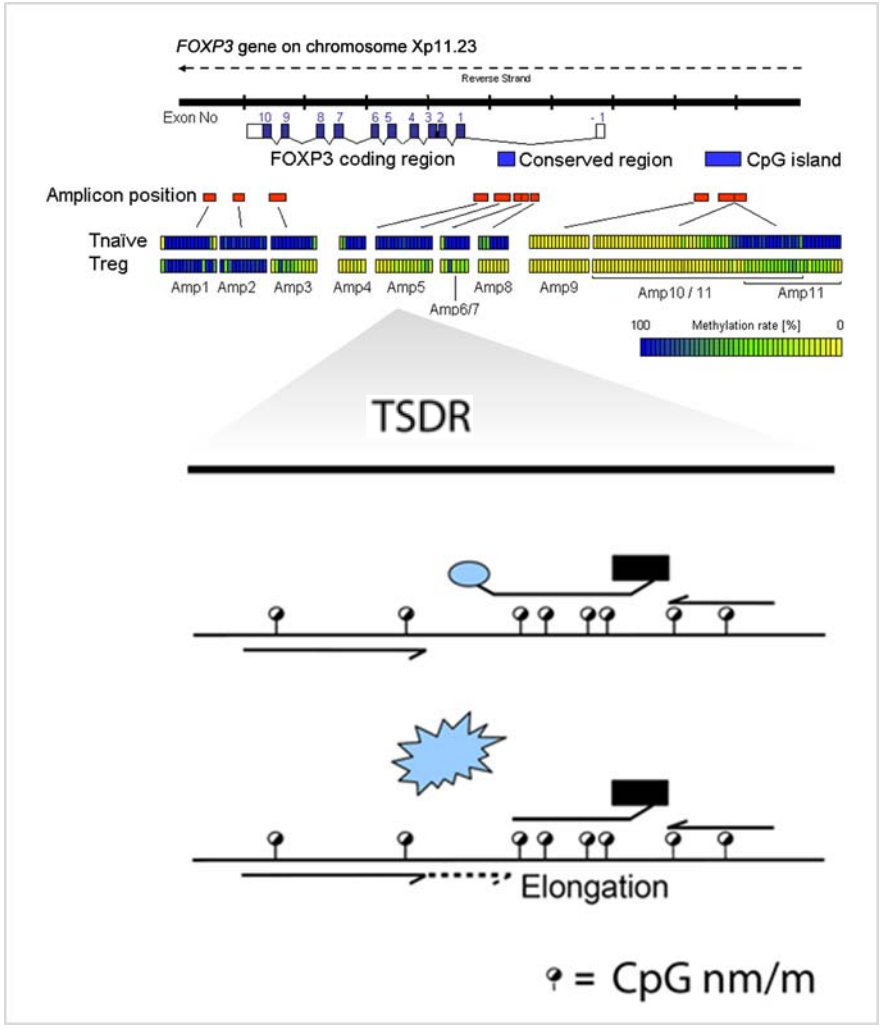
Development of qPCR Assay for Treg Specific, Demethylated Region (TSDR) of FoxP3:



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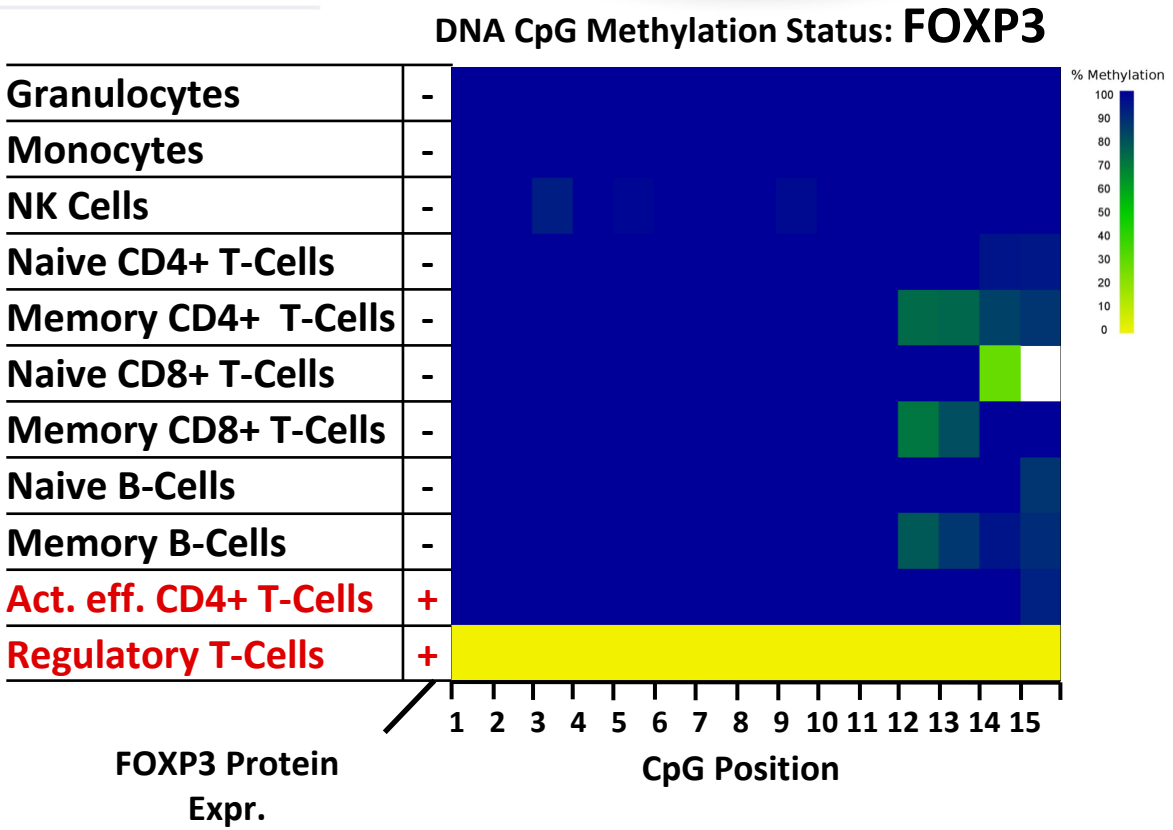
Wieczorek et l., Cancer Res. 2009 Jan 15;69(2):599-608.

Epigenetic Status of the Foxp3 Gene – Ideal Marker for Regulatory T Cells

Epigenetic activation of Foxp3

- Is solely observed in regulatory T cells
- All other analyzed leukocytes and tissues are epigenetically inactive
- Accurate Treg counting is feasible



Foxp3-Expression

- Occurs transiently also in non-regulatory activated effector CD4+T cells
- FOXP3 expression based measurements (e.g. FACS) represent a mixed count of regulatory as well as activated T-cells. Accurate Treg counting is not feasible.

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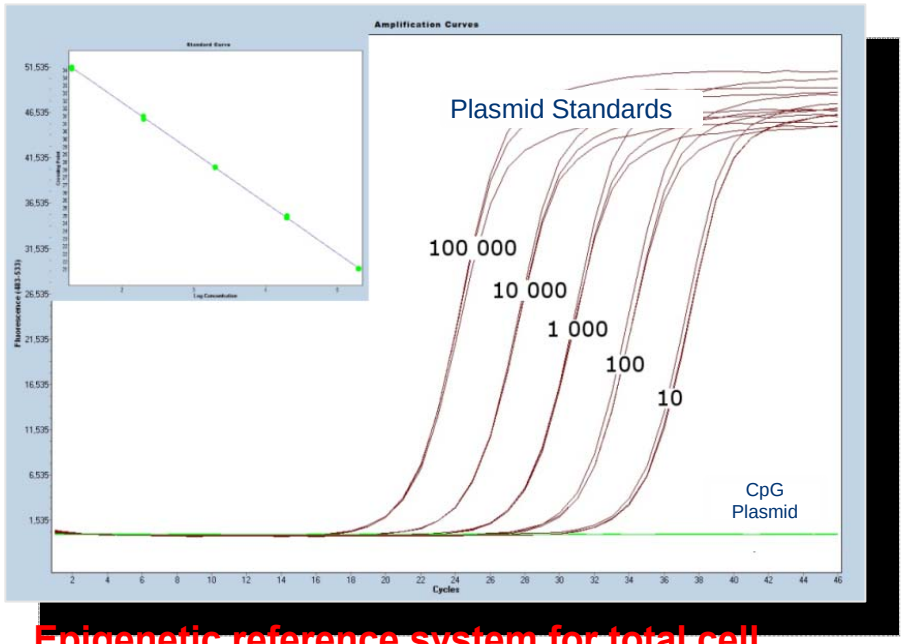
Baron et al., Eur. J. Immunol. 2007. 37: 2378-2389

Quantitative, Highly Sensitive Foxp3 Assay

Epigenetic Foxp3 qPCR Assay

- Very robust and reproducible real time-PCR test
- Sensitive and quantitative for measurements of physiological and pathological regulatory T cell levels
- Application for immune monitoring
  - Cancer
  - Autoimmune disease
  - Transplantation/immune suppression

PCR specific to TpG



Epigenetic reference system for total cell number determination

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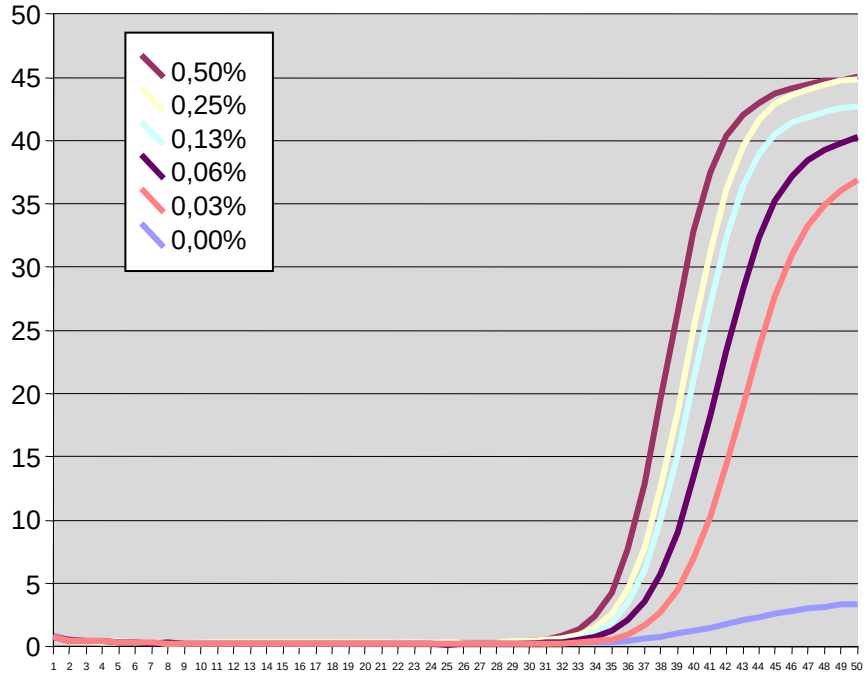
Wieczorek et l., Cancer Res. 2009 Jan 15;69(2):599-608.

Very Sensitive Detection of Small Cell Numbers (Foxp3-Marker)

Spiking Experiment: small amount of Treg in large background of granulocytes

- 0.50% (56 Tregs)
- 0.25% (28 Tregs)
- 0.13% (14 Tregs)
- 0.06% (7 Tregs)
- 0.03% (3 Tregs)
- 0.00% (0 Tregs)



Each in presence of 12,000 granulocytes

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Wieczorek et al., Cancer Res. 2009 Jan 15;69(2):599-608.

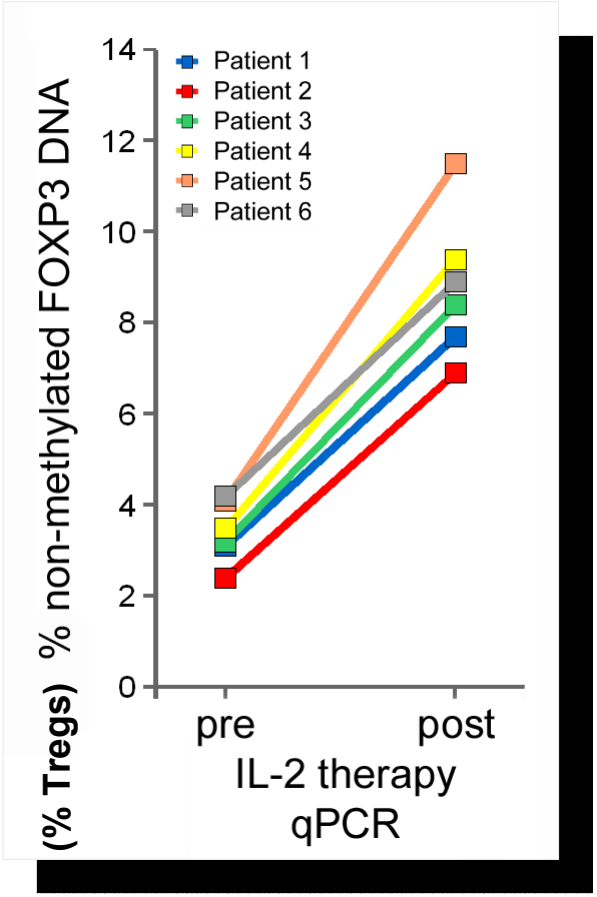
Application for Clinical Monitoring

Epigenetic Foxp3-Analysis Shows:

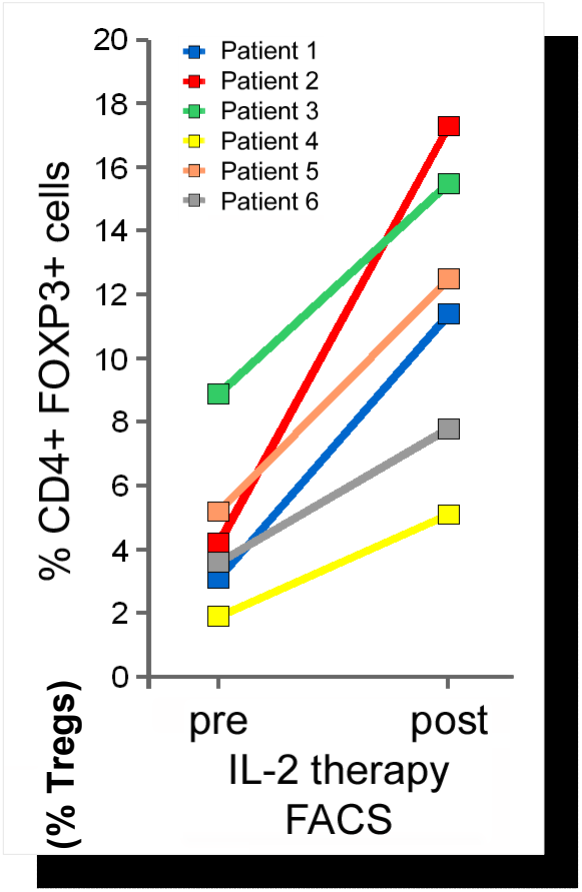
- Same trend of cell numbers
- Less variation

Measured in PBMCs of melanoma patients before and after IL-2 treatment.

Epigenetic qPCR
(methylation FOXP3 gene)



FACS
(antibody FOXP3 protein)



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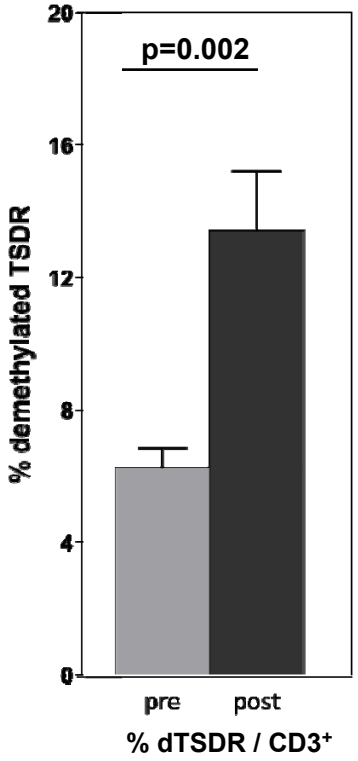
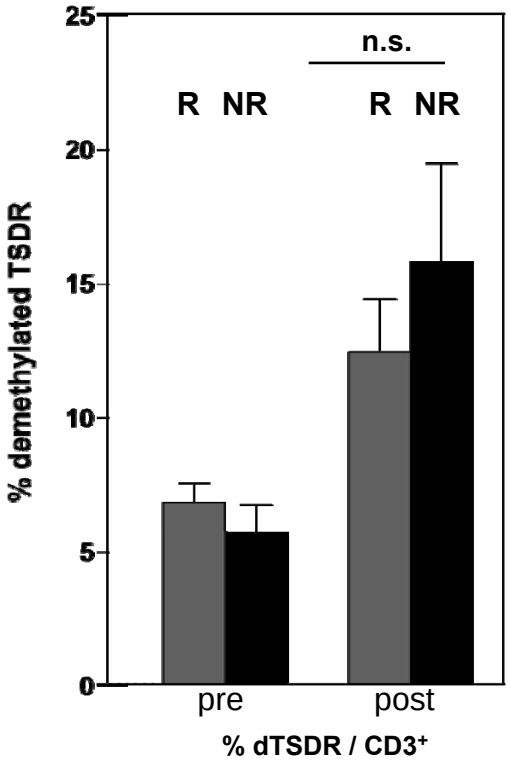
Wieczorek et al., Cancer Res. 2009 Jan 15;69(2):599-608.

Treg Monitoring during mRCC Therapeutic Vaccination

- Vaccination increases Treg counts
- Stronger increase in non-responders





Schwarzer, et al., Regulatory T-cells and associated pathways in metastatic Renal Cell Carcinoma (mRCC) Patients undergoing DC-vaccination and cytokine-therapy, 2012 *submitted*

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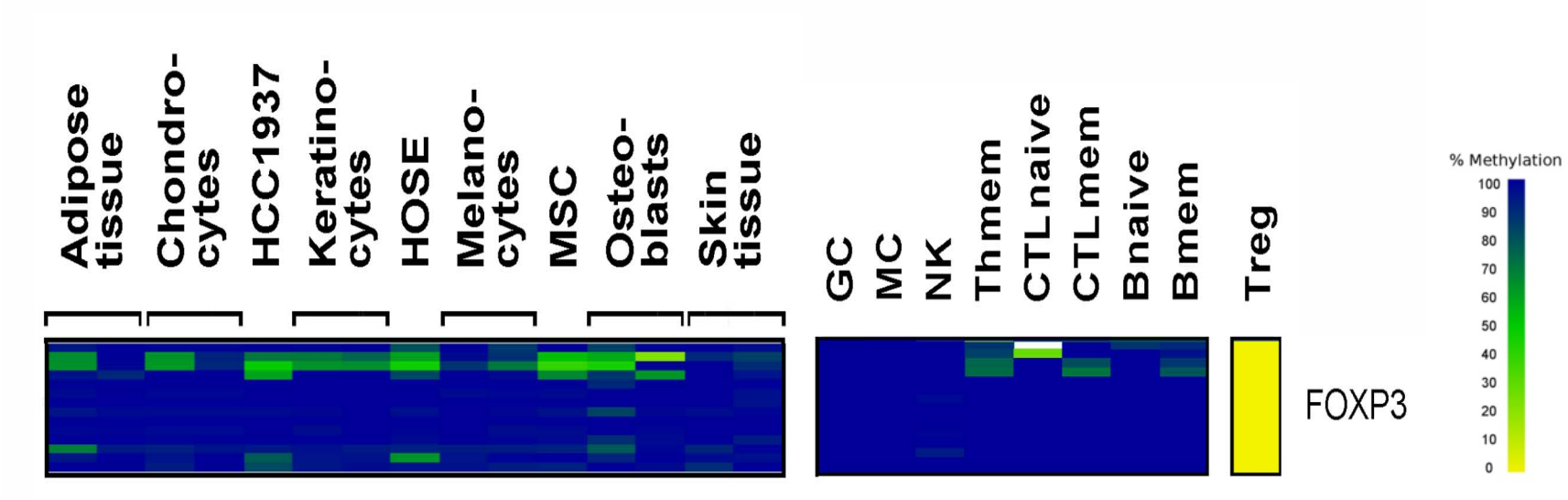
Epigenetic FOXP3 Marker Application for Tissue Samples

Epigenetic Foxp3 marker is unique for Tregs compared to all analyzed tissues

- Epigenetic tests can also be used to detect Tregs in solid tissues
- Alternative to Immunohistochemistry
- Delivery of Quantitative Results



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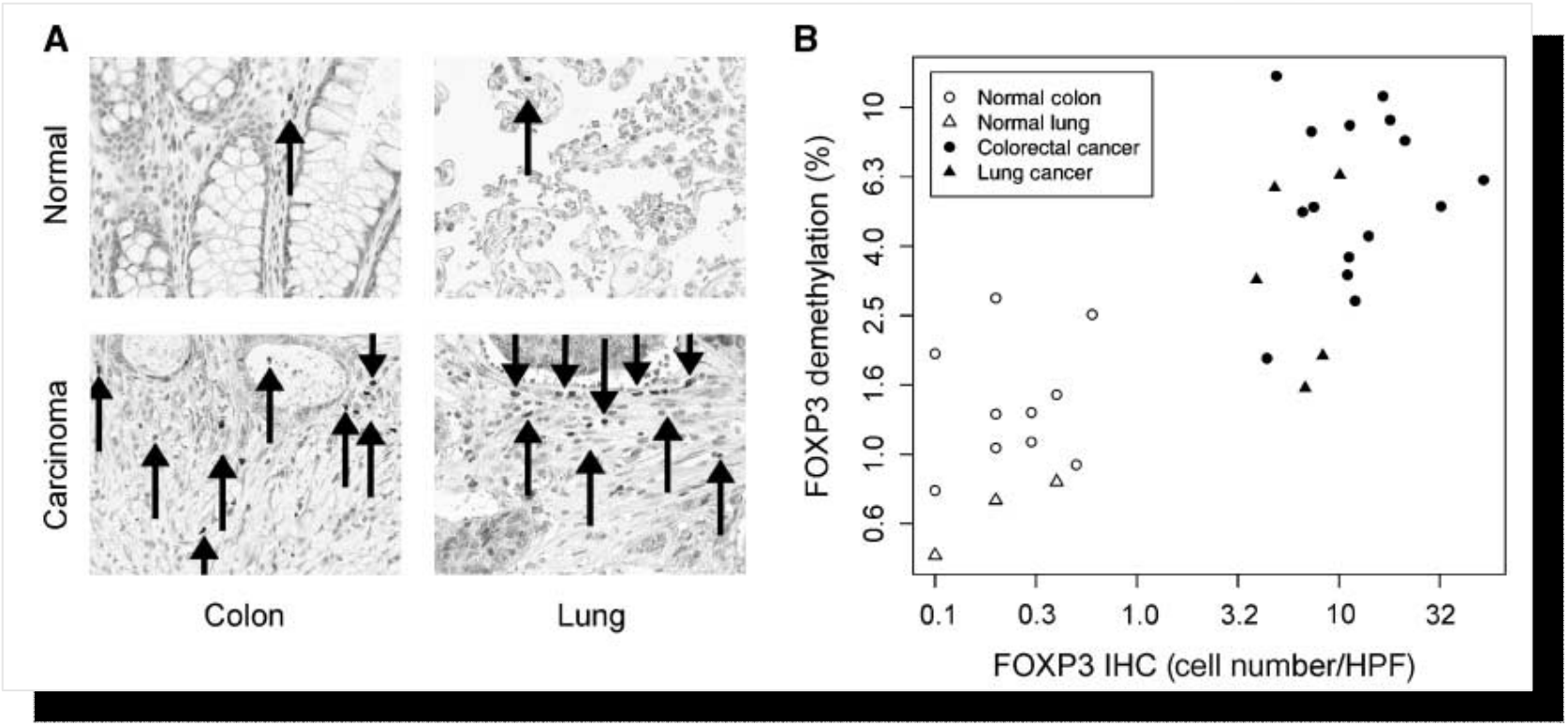
Wieczorek et al., Cancer Res. 2009 Jan 15;69(2):599-608.

Comparison of Treg Immunohistochemistry and Epigenetic qPCR



- Epigenetic quantitation of cells (Treg) can be performed using paraffin embedded tissue samples.
- Cell counts obtained by the two methods correlate

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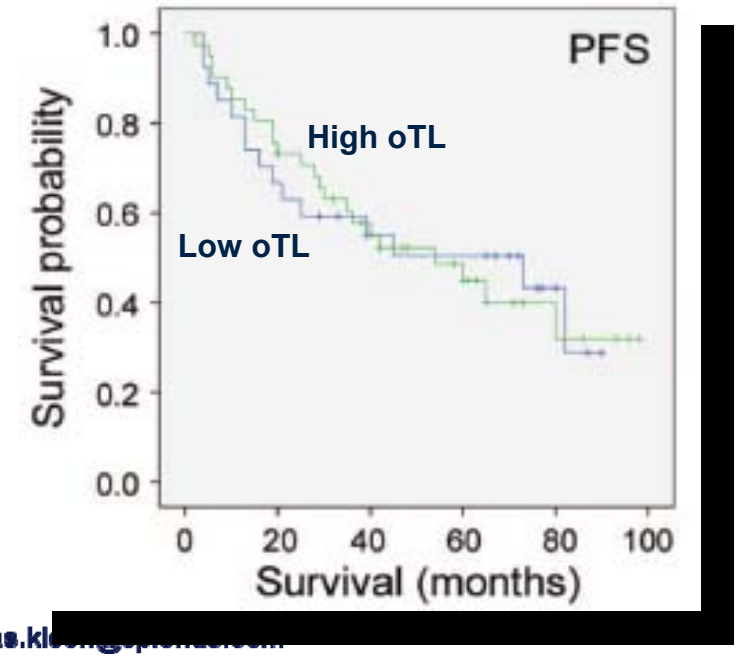
Wieczorek et al., Cancer Res. 2009 Jan 15;69(2):599-608.

Comparison of oTL (CD3+) Immunohistochemistry and Epigenetic qPCR

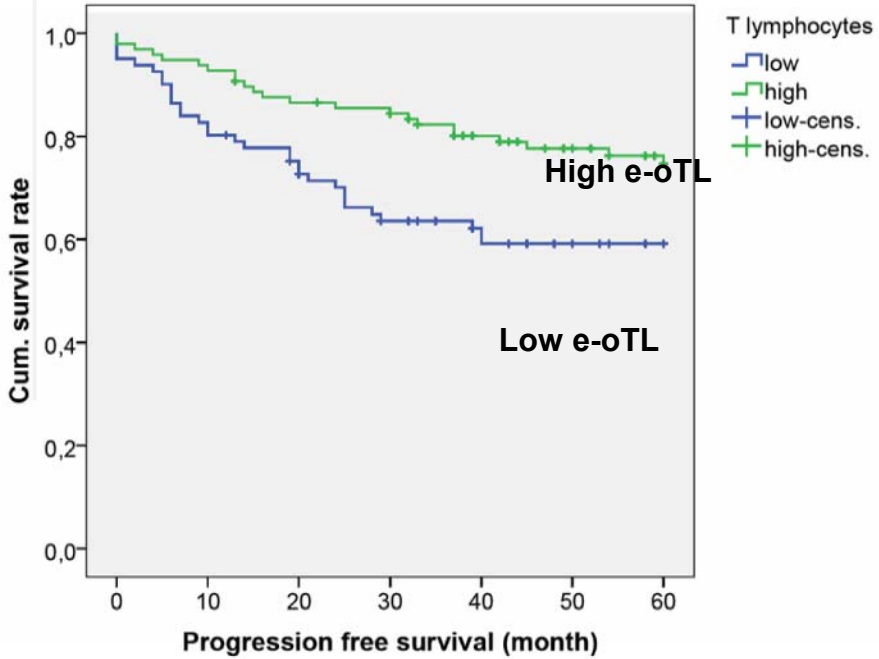
- Classic Immunohistochemistry for oTL done by Pathologist for colorectal cancer patients (n=149)
- **Separation into high and low oTL shows no difference in prognosis**

- Epigenetic analysis of same tumor tissue samples
- **Separation into high and low oTL shows difference in prognosis**

Immunohistochemistry



Epigenetic oTL Assay



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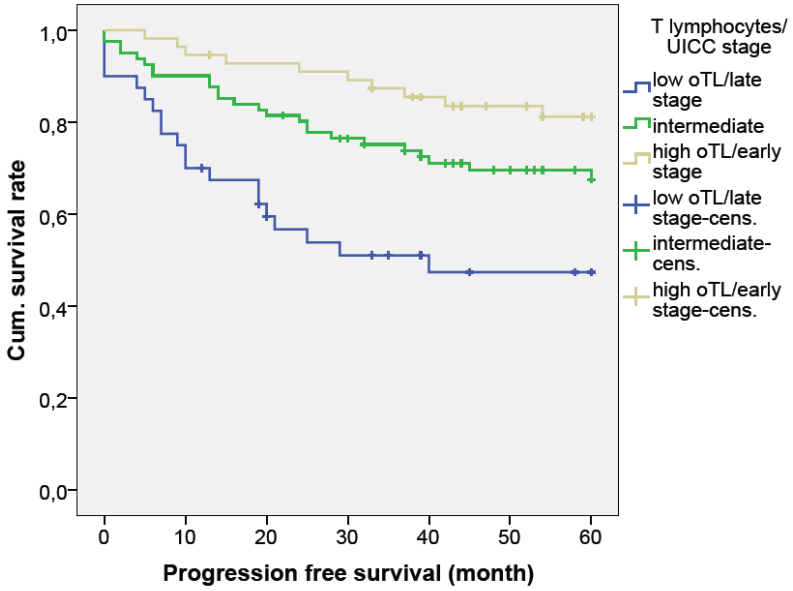
Combination of Scoring Improves Prognostic Value

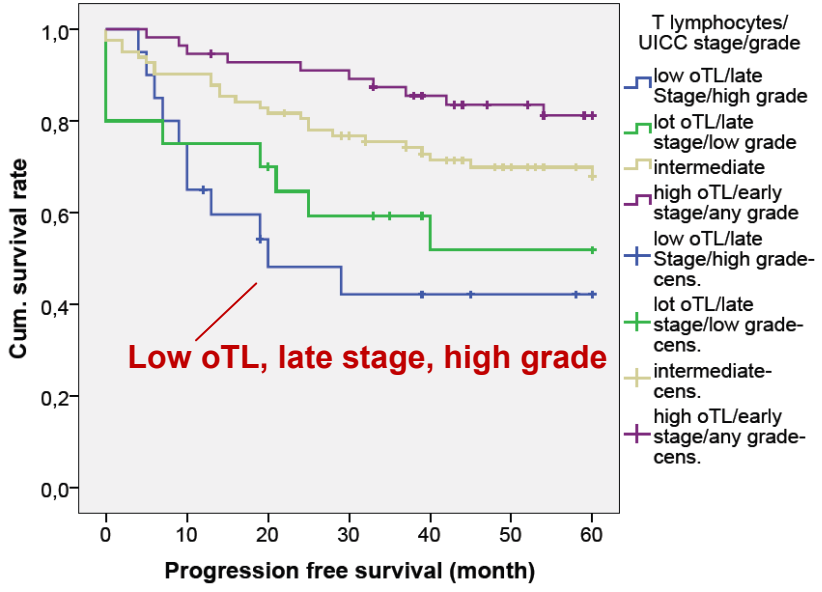
- Coupling oTL with UICC tumor staging
- Further separation into prognostic groups possible?

- Coupling oTL with UICC stage and with UICC tumor immunhistology-Grading
- Additional separation into prognostic groups shows more detailed information

Score oTL + UICC



Score oTL + UICC + Grading



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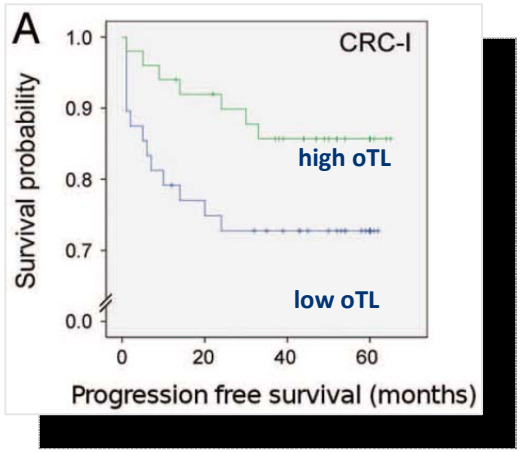
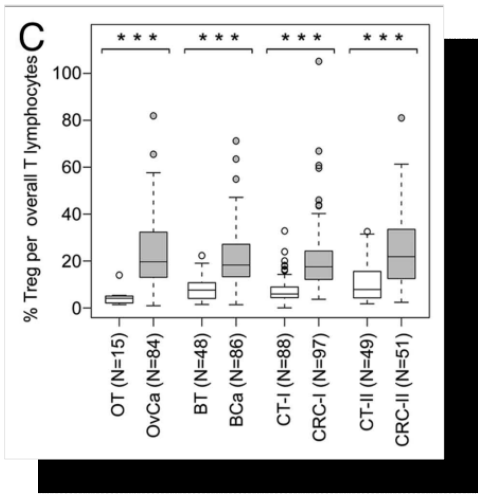
Prognostic Value of Epigenetic oTL (CD3) and Treg Count

Tumor Infiltrating Lymphocytes in Ovarian, Breast Cancer and Colorectal Carcinoma





- Epigenetic qPCR assay for CD3 uncovers overall T cell (oTL) counts in tissue
- High oTL counts in colorectal and ovarian tumor correlate with better survival
- Ratio of Treg/CD3 cells highly elevated in tumor vs. healthy tissue (3-8% vs 18 25%) throughout various tumor entities suggest it is a consistent defect of the tumor associated immune status
- Tested in ovarian, lung and colorectal (616 samples)

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Sehouli et al., Epigenetics 2011 Feb 6:2, 236-246

Standardization and Comparison to Flow Cytometry and Immunohistochemistry

Standardization of epigenetic qPCR

- Intra assay CV ≤ 15%
- Inter assay CV ≤ 20%
- Internal plasmid standards and reference sample on each plate
- No immediate sample prep needed. Collected sample can be stored and shipped at -20°C/dry ice w/o stability limitation

Flow Cytometry

- Equivalent numerical results are obtained by flow cytometry and epigenetic qPCR
- Less arbitrary/subjective bias (e.g. gating settings, PBMC prep in flow)

Immunohistochemistry

- No subjective definition of Invasive margin (IM) necessary with epigenetic qPCR
- Results will as well be depending on region and number of biopsies taken

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Summary for Tumor Microenvironment

- Composition of tumor cell in Microenvironment can be quantitatively assed by epigenetic measurements
- mRNA can not be associated to cell number since overall amount of transcript varies and mRNA is much less stable the DNA
- FACS requires single cell suspension and IHC is semi quantitative
- Treg-to-oTL Composition is disturbed/dysbalanced in solid tumors potentially indicating fundamental mechanism of tumor evasion
- Risk for recurrence or death decreases 0.2 %to 3.4% for each 1% increase in oTL in tumor environment as does PFS and OS increase with oTL increase
- Epigenetic quantification of T cells and others could serve as independent or combinatorial clinical parameter for outcome prognosis

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"Introducing the Next Generation of Cell Mediated Immune Monitoring!"

Goal of the Epigenetic Technology

- Further increase the number of Biomarkers complementary and based on FACS, IHS and mRNA
- Enable subsequent implementation of such additional Biomarker monitoring during large clinical Phase 2 and Phase 3 studies based on in inherent assay characteristics
  - Small volume of sample needed
  - Freeze/Thaw stability
  - Sensitivity
  - Standardization
  - Logistics friendly
- Potential application as Companion Diagnostic

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References and Bibliography

- Baron et al., Epigenetics 2006 1(1):55-60: DNA methylation analysis as a tool for cell typing.
- Floess et al., PloS 2007 5(2):e38: Epigenetic control of the foxp3 locus in regulatory T cells.
- Baron et al., Eur J Immunol 2007 37(9): 2378-89: DNA demethylation in the human FOXP3 locus discriminates regulatory T cells from activated FOXP3(+) conventional T cells.
- Polansky et al., Eur J Immunol 2008 38(6):1654-63: DNA methylation controls Foxp3 gene expression.
- Wieczorek et al., Canc Res 2009 69(2):599-608: Quantitative DNA methylation analysis of FOXP3 as a new method for counting regulatory T cells in peripheral blood and solid tissue.
- Stockis et al., Eur J Immunol 2009 39(3):869-82: Comparison of stable human Treg and Th clones by transcriptional profiling.
- Hoffman et al., Eur J Immunol 2009 39(4):1088-97: Loss of FOXP3 expression in natural human CD4+CD25+ regulatory T cells upon repetitive in vitro stimulation.
- Schaub et al., J Allergy Clin Immunol 2009 123(4): 774-82: Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells.
- Nunzio et al., Blood 2009 04-214593: Wild-type FOXP3 is selectively active in CD4+CD25hi regulatory T cells of healthy female carriers of different FOXP3 mutations.
- Liu et al., PLoS ONE 2010 5 10 e13267 T Regulatory Cells in Cord Blood—FOXP3 Demethylation as Reliable Quantitative Marker.
- Brusko et al., PLoS ONE 2010 5 7 e11726 Human Antigen-Specific Regulatory T Cells Generated by T Cell Receptor Gene Transfer.
- Sehouli et al., Epigenetics 2011 Epigenetic quantification of tumor-infiltrating T-lymphocytes.
- Steinfelder et al. Blood 2010, Epigenetic modification of the human CCR6 gene is associated with stable expression of CCR6 in T cells.

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BACKUP SLIDES

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Epiontis

Innovative CRO and Biotech Company:

- Founded in 2003
- Headquartered in Berlin, Germany with global services
- Funded by revenues generated through CRO activities, government grants, business angels, and founders

Epiontis' History:

- Initial license of epigenetic technology for cell characterization 2003
- Beginning of in-house IP generation 2003
- Established laboratories for molecular biology and cell biology 2003
- Adapted technology for regenerative medicine 2004
- Major Industry collaborations since 2005
- Adapted technology for Immune Monitoring 2007
- Engaged in Clinical Trials since 2008

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Scientific Rationale for CMI Monitoring

- Cell Mediated Immunity (CMI) is a critical component during most immunological responses; involved in cancer, autoimmunity, allergic responses, and infectious diseases

- During Immunotherapy approaches of malignancies and autoimmune diseases CMI characterization is advisable

- Reliance on antibody response as correlates of protection is often not sufficient for high value target pathogens with unmet medical need (e.g., HIV, HCV, TB, Smallpox)

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Regulatory Rational for CMI Monitoring

- Adding immunological endpoint analysis to clinical endpoints provides mechanistic information and early developmental guidance
- Regulatory agencies, including the EMA and U.S. FDA, encourage better prognostic models and establishment relevant biomarkers for immune modulation and immunotoxicity
- It is advisable to include CMI monitoring as adjunct to clinical trials, including biologic agents and vaccines
- CMI monitoring is crucial as analytic and diagnostic component during development of immune modulators and will be essential for their routine clinical implementation

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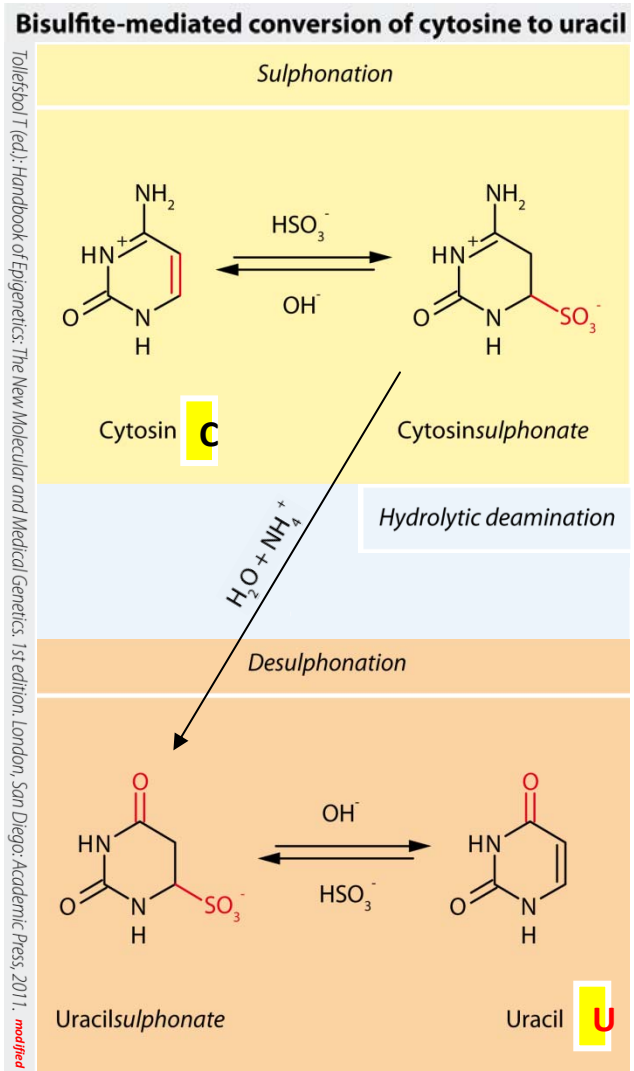
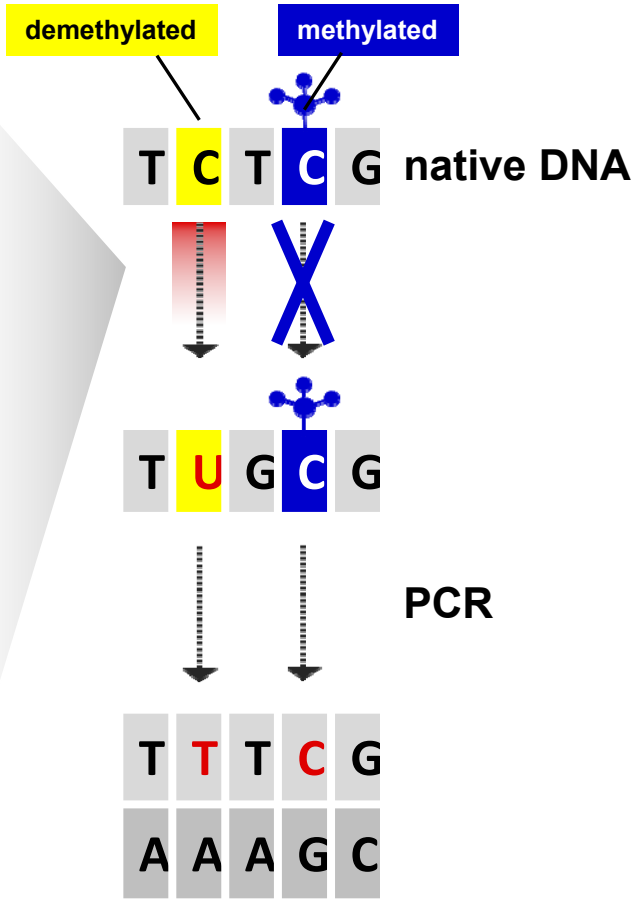
Validation and Routine Testing Technology
Bisulfite Conversion:





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Comparison Whole Blood versus PBMC Sample Testing

Biological variability

- Whole blood and PBMC samples from 5 different donors were tested in parallel

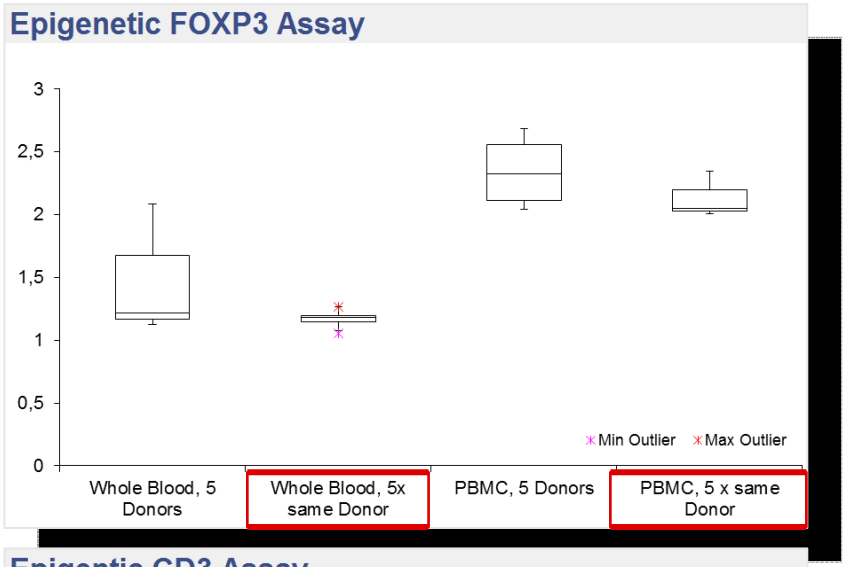
➢ PBMC samples show expected higher cell numbers due to separation process enrichment

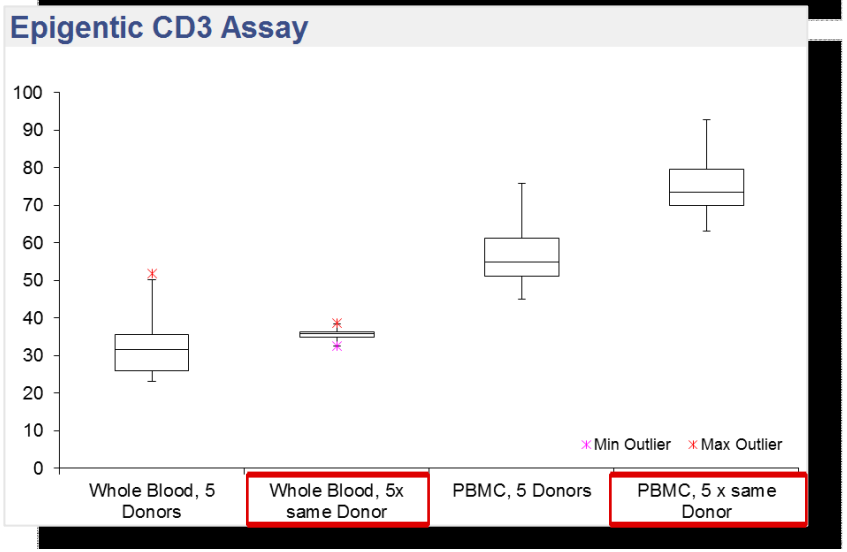
Technical variability

- 5 whole blood samples from the same donor and five independent PBMC preparations thereof were tested in parallel

➢ Additional PBMC preparation introduces higher variation

➢ Whole blood is preferable form of sample





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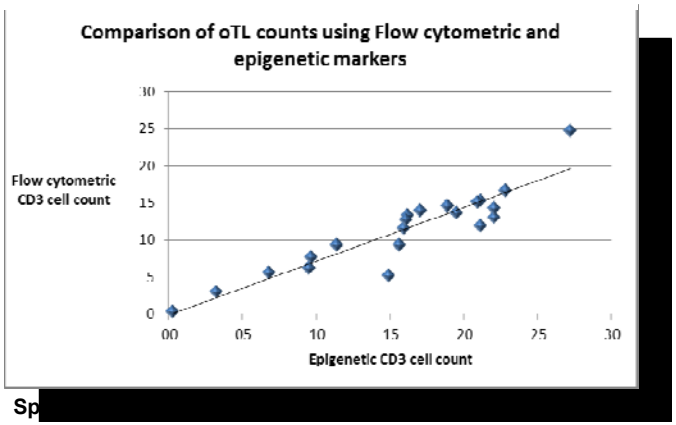
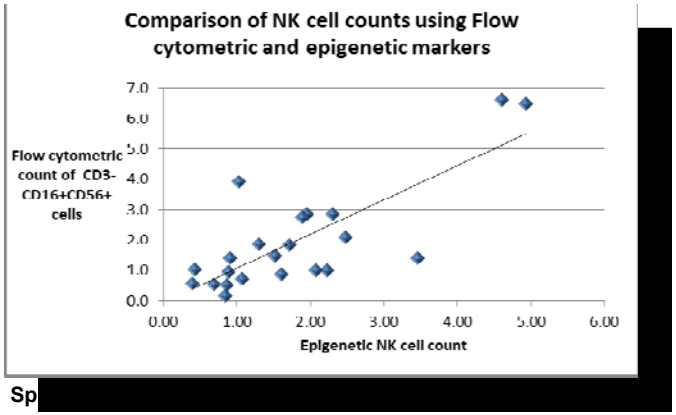
Additional Assays - Correlation Epigenetic qPCR and Flow Cytometry

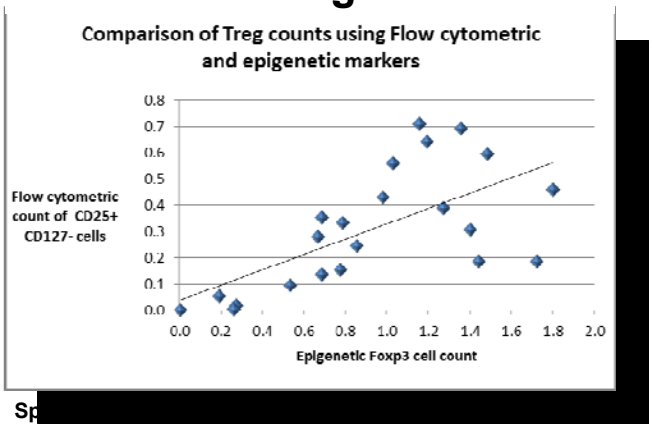
Overall T cells



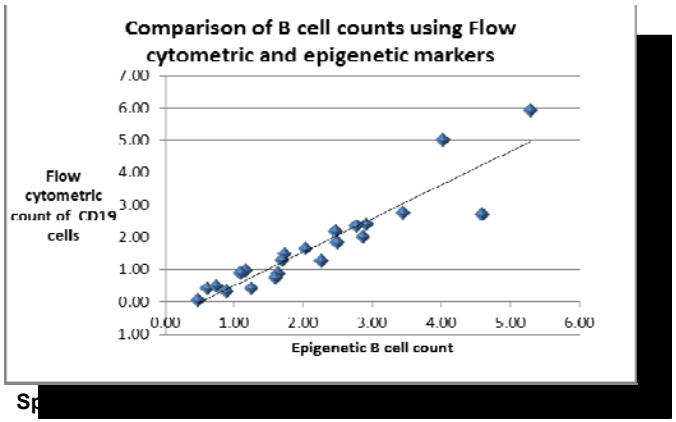
NK cells



Treg



B cells



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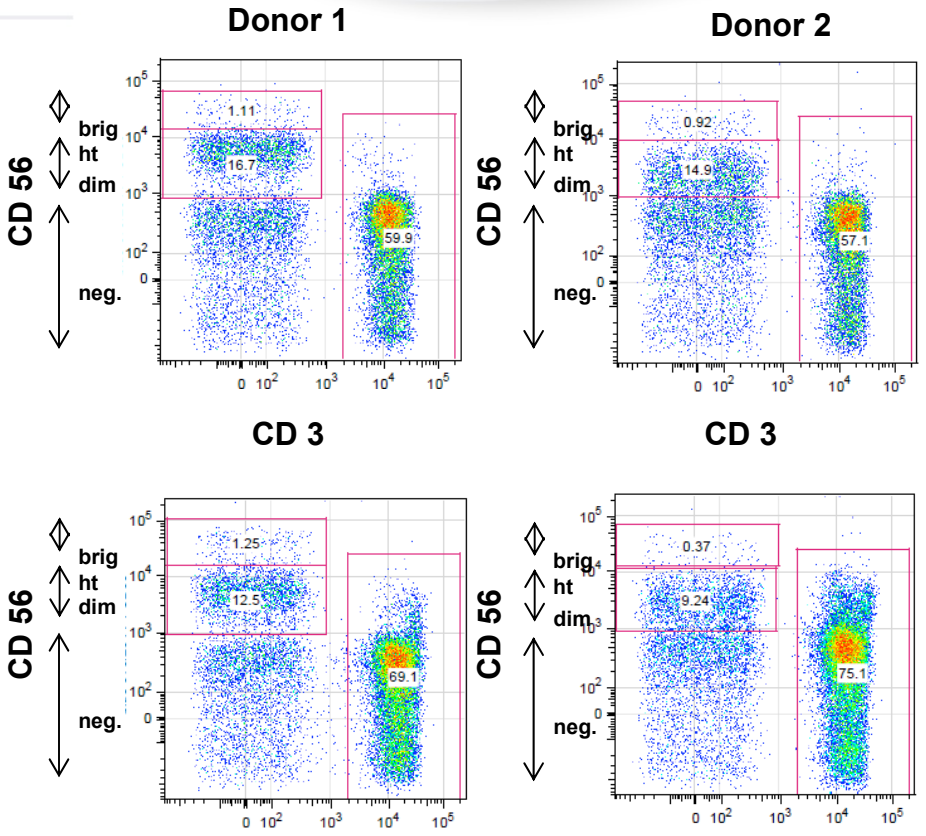
Epigentic Selective Specificity for CD56+NKdim (cytotoxic) cells

Cell type	Epiontis' NK^{dim} marker [%]
CD8+ T-cells	1.35
CD4+ memory T-cells	0,0
CD4+ naive T-cells	0,0
CD4+ T-cells (all)	0,1
CD14+ monocytes	0.1
CD15+ granulocytes	0.0
CD19+ B-cells	0.0
CD3- CD56++ Nk^{bright} cells	**0.1**
CD3- CD56+ Nk^{dim} cells	**88.8**
CD3+ CD56+ NK T-cells	3.2
CD56 depleted CD8	4.4
CD3+ CD8+ CD45RA+ naive T cells	0,0
CD8 effector memory	0,0
keratinocytes	0,0
cartilage	0,7
osteogenic iMSC	0,3
chondrogenic iMSC	0,5
lung carcinoma cell line	0,0



- Only CD56 dim NK cells (cytotoxic) are detected
- CD56 bright NK cells (cytokine secreting) are not detected

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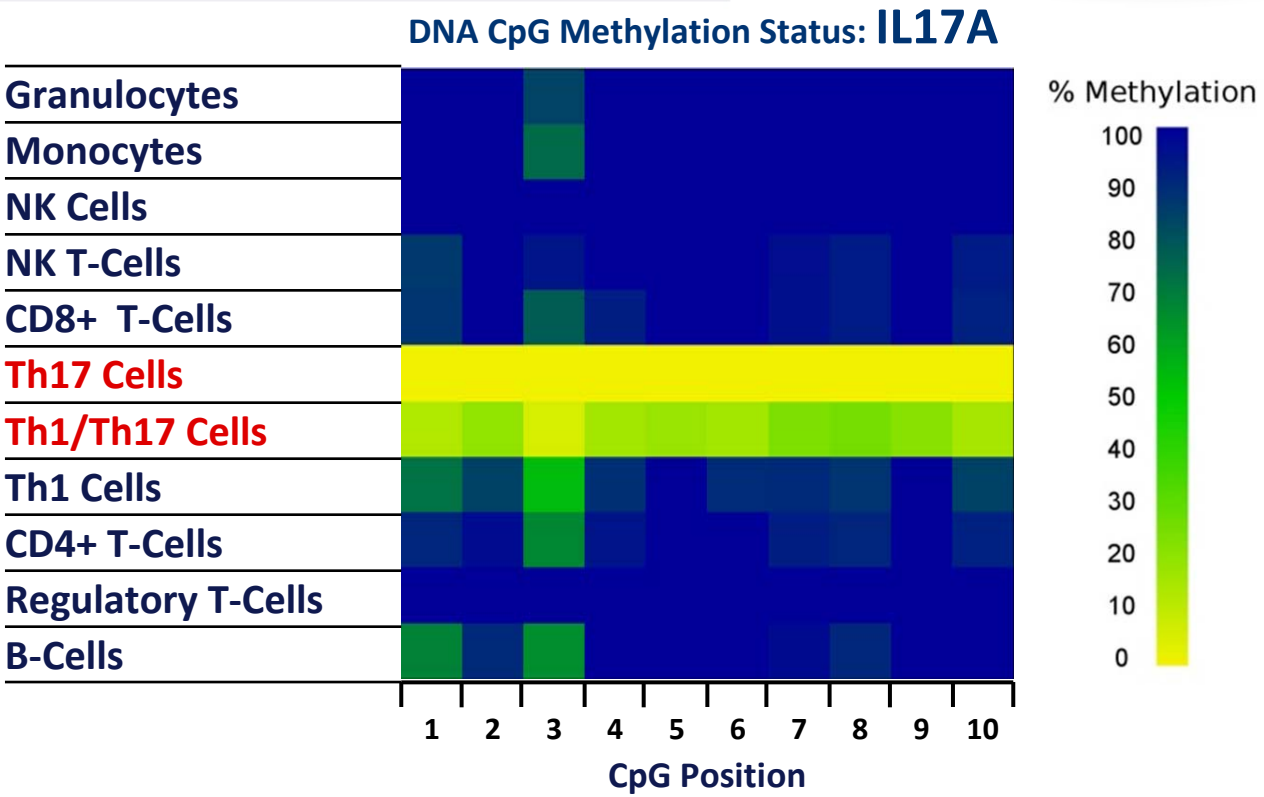
Epigenetic Th17-Assay



- Is solely observed in Th17 cells and Th1/Th17 (IFN-g+/IL17+ double positive) cells
- All other analyzed leukocytes and tissues are epigenetically inactive

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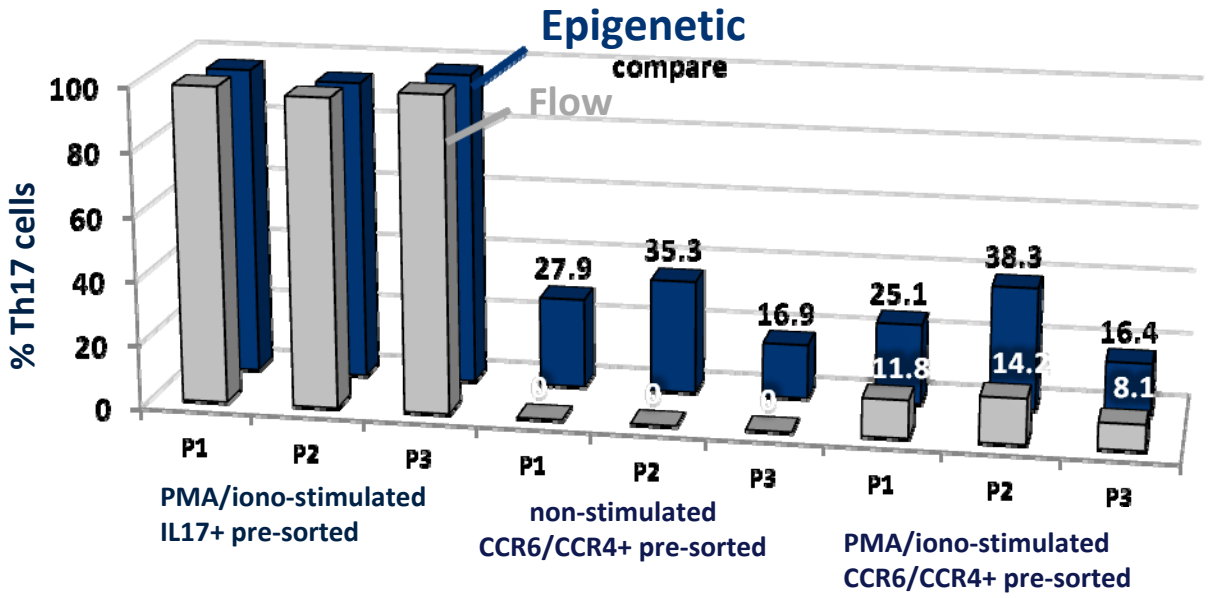
Epigenetic Th17-Assay versus Flow



- All Th17 cells detected by flow also detected by epigenetic assay
- Epigenetic assay detects identical cell counts without prior stimulation (lineage commitment detection?)
- Flow cytometry detects only 50% of epigenetic signal in stimulated CCR6/CCR4+ sorted cells (incomplete stimulation, Flow detection threshold?)

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Monitoring Change in Treg-Levels During Drug Therapy induced Immune Suppression

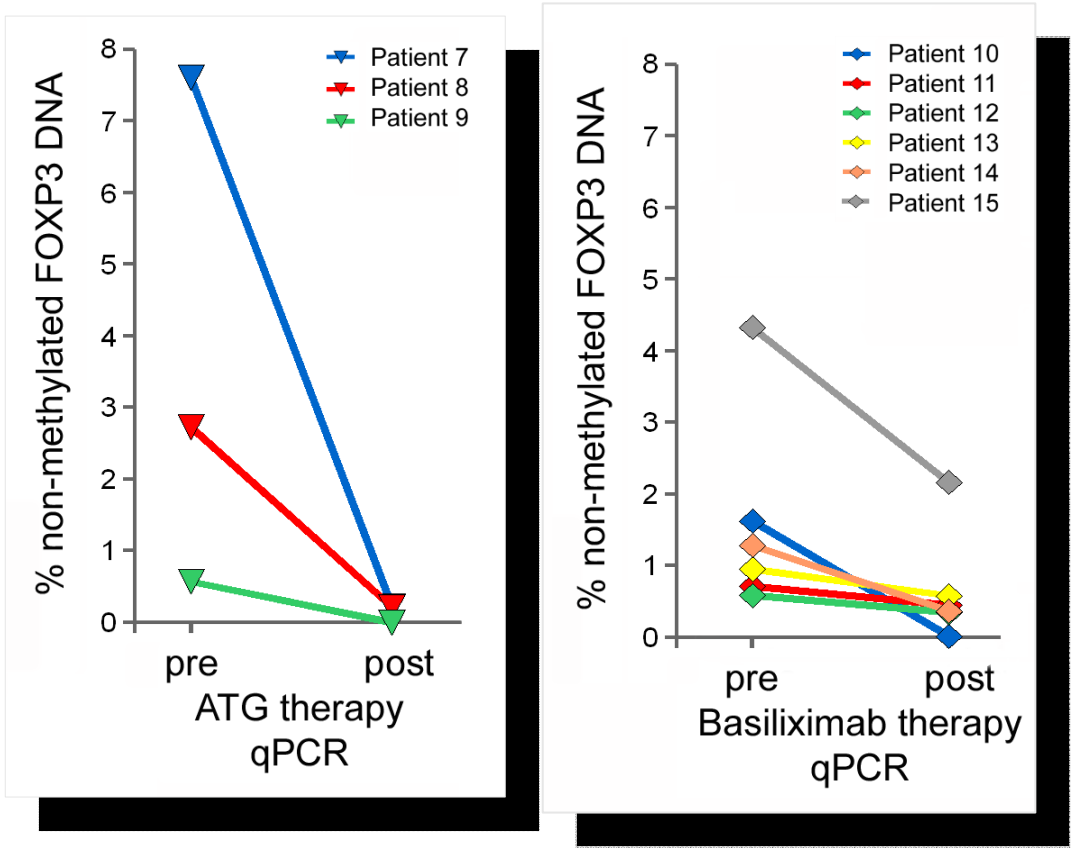
Immunosuppressive treatment after transplant

- Thymoglobulin (ATG, polyclonal antibodies for T-cell depletion, Genzyme) in combination with tacrolimus, mycophenolate mofetil and steroids

- Basiliximab (anti IL2R, Novartis)

Detection of Immunosuppressive treatment with In all monitored patients, Treg levels dropped in periphery, which was measured by the FoxP3-Methylation-Test

Epigenetic qPCR
(methylation FOXP3 gene)



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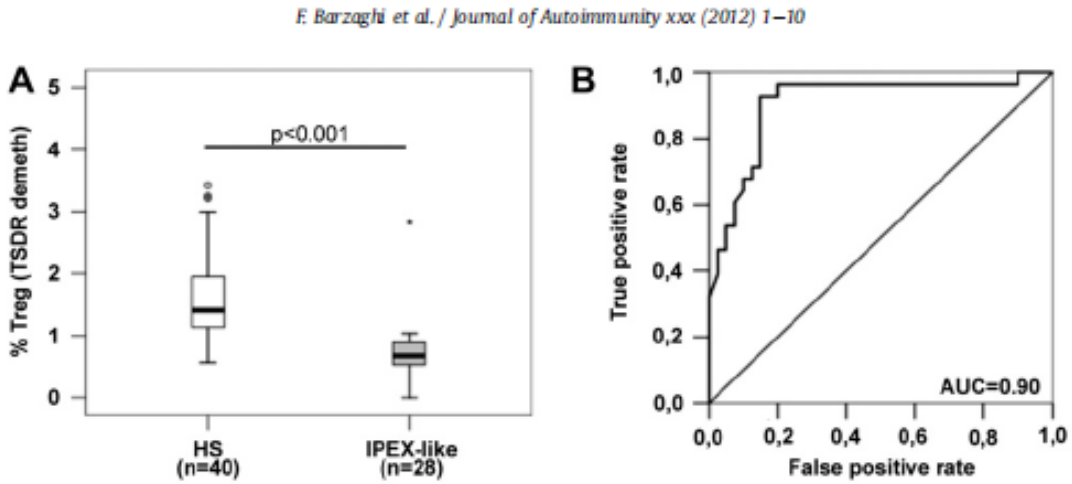
Wieczorek et al., Cancer Res. 2009 Jan 15;69(2):599-608.

Diagnosis of IPEX-like Syndrom (epigenetic FOXP3 Assay)

- IPEX-like patients have significantly lower Treg counts
- Epigenetic PCR: good specificity/sensitivity profile (90% AUC in ROC curve)
- Not possible with flow cytometry due to noise



Barzaghi F, et al., Demethylation analysis of the FOXP3 locus shows quantitative defects of regulatory T cells in IPEX-like syndrome, Journal of Autoimmunity (2012), doi:10.1016/j.jaut.2011.12.009

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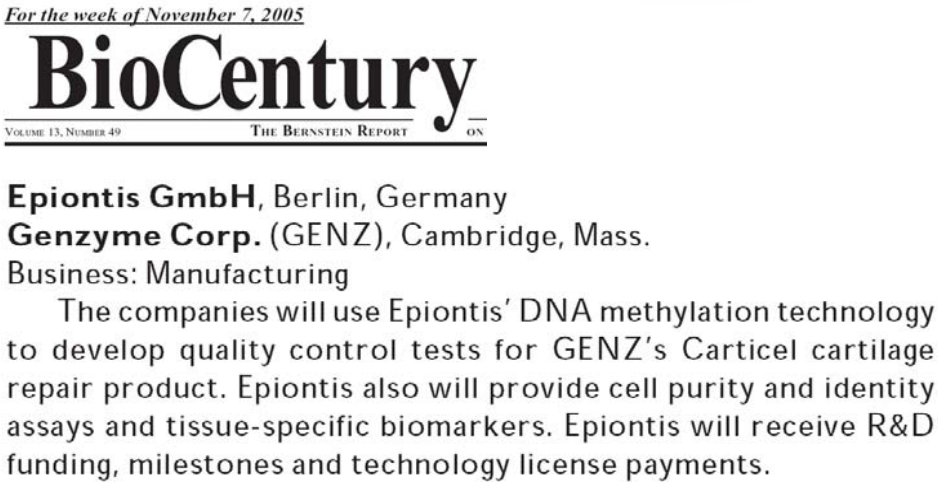
Commercial Applications:

Standard Release Test for Genzyme's Carticel Product



For the week of November 7, 2005

BioCentury

VOLUME 13, NUMBER 49 THE BERNSTEIN REPORT ON

Epiontis GmbH, Berlin, Germany
Genzyme Corp. (GENZ), Cambridge, Mass.
Business: Manufacturing

The companies will use Epiontis' DNA methylation technology to develop quality control tests for GENZ's Carticel cartilage repair product. Epiontis also will provide cell purity and identity assays and tissue-specific biomarkers. Epiontis will receive R&D funding, milestones and technology license payments.

Quantitative Assay for Immune Cells

- For QC of therapeutic cells
- For clinical trial immunomonitoring in patient samples

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