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Anti-Biopharmaceutical Immunization: Prediction and analysis of clinical relevance to minimize the risk

“Goals and Update”

Marc Pallardy, INSERM UMR 996, France (IMI JU managing entity)
Dan Sikkema, GSK (Overall Project Coordinator)

EIP Meeting, München, 2013

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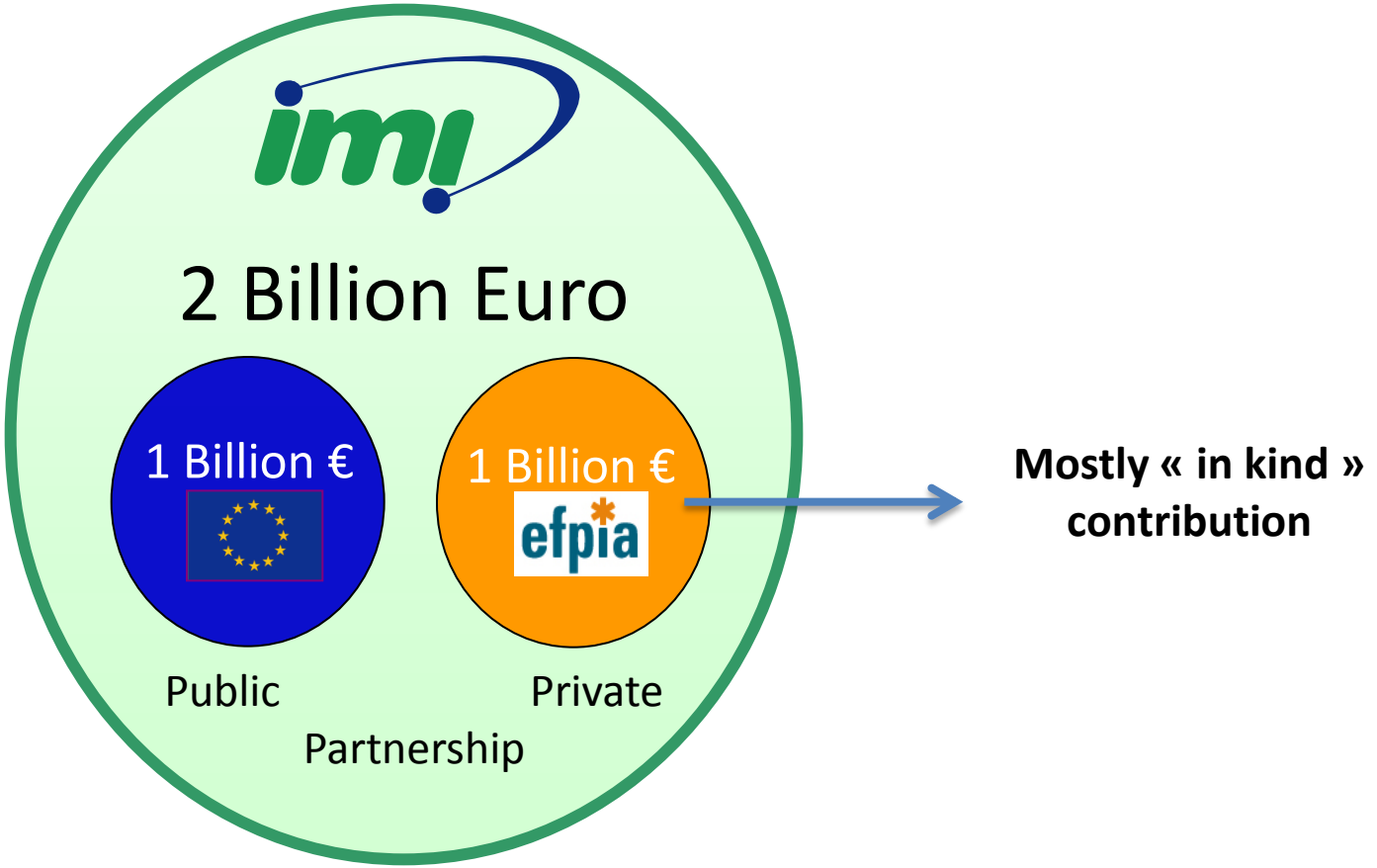
Innovative Medicines Initiative

A public-private partnership
focused on needs common to
pharmaceutical industry and patients

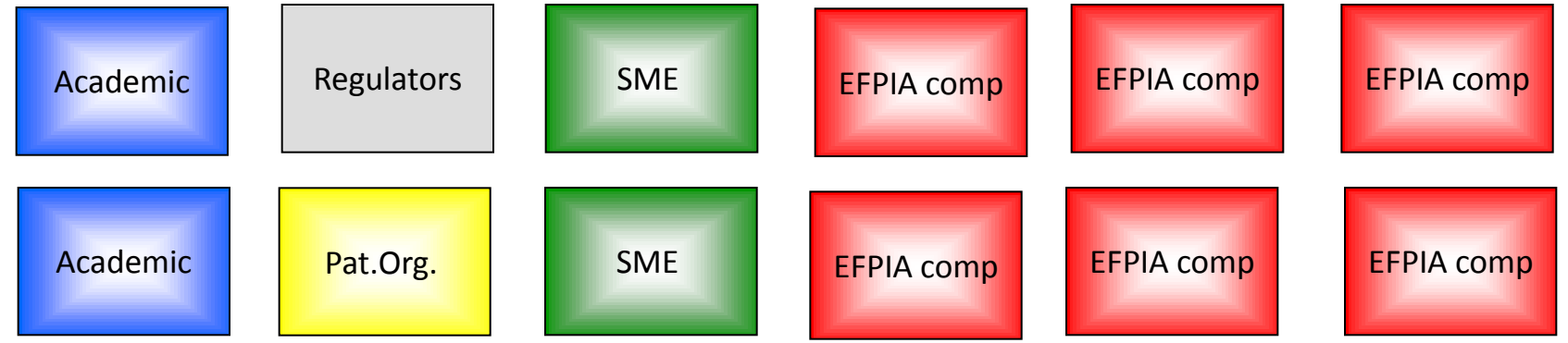
efpia

Innovative Medicines Initiative: the Largest PPP in Life Sciences R&D



EFPIA = European Federation of Pharmacological Industries and Associations

Overall Structure of Research Projects



"Applicants consortium"

IMI beneficiaries

"EFPIA consortium"

EFPIA *in kind* contribution

(no public funding)

Start date: March 1st 2012

5 years

Total project cost €34.9 million

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- **EFPIA member companies: 9 (2 more in discussion for entry)**
- **Academic Partners: 25**
- **SMEs: 2 (3 in April)**
- **36 partners total**

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Objectives and driving forces (1)

- **Access to large cohort of patients treated with different BPs**
  - Hemophilia A
    - Factor VIII
  - Multiple Sclerosis
    - Interferon beta, Natalizumab
  - Inflammatory diseases
    - Rheumatoid Arthritis
      - Infliximab, Adalimumab, Rituximab, Etanercept
    - SLE
      - Rituximab
    - Inflammatory Bowel Disease
      - Infliximab, Adalimumab

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Objectives and driving forces (2)

- **Validation of ADA assays**
  - Assay standardization, SOP for each assay, internal review of the validation protocols
  - Universal assay validation protocol
  - Characterization of ADA in prospective and retrospective patients' cohorts
  - Generation of an internal standard (ADA of human origin)
- **Novel approaches to characterize AD lymphocyte responses**
  - Retrospective and prospective patient samples (PBMC, serum, DNA, RNA)
- **Development and validation of innovative prediction tools**
- **Collection and integration of immunogenicity-related data and clinical relevance of ADA :** unique data base
  - Collect a single data bank with all the information gathered during the program
  - Develop statistical models to analyze heterogeneous type of information: integration
  - Predictive signatures for immunogenicity phenotypes and immunogenicity-related clinical events (we hope !)

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Work Packages

- WP1 “ADA assay development and validation and cohort management”
  - **F. Deisenhammer, Claire Holland, Claudio Carini**
- WP2 “Cellular characterization and mechanisms of the AD immune response”
  - **C. Mauri, H. Kirby, V. Mikol**
- WP3 “Evaluation and development of technologies for predicting immunogenicity”
  - **B. Maillère , S. Spindeldreher, Ch. Ross-Pedersen**
- WP4 “Establishment of a data base, data analyses and integration”
  - **J. Davidson, Ph. Broet, A. Hincelin-Maury**
- WP5 “Project management and communication”
  - **R. Bertini, Dan Sikkema, M. Pallardy**
- Cohort management: Cohort leaders
  - **Rheumatoid Arthritis: X. Mariette, Inflammatory Bowel Disease: M. Allez; Hemophilia: J. Oldenburg; Multiple Sclerosis: A. Fogdel-Hahn**

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Patient Cohorts

- **Inflammatory diseases: Kremlin-Bicêtre (Xavier Mariette) (15 French centers)**
  - University College, London (Claudia Mauri)
  - Leiden University Medical Center, Leiden (Tom Huizinga)
  - University of Amsterdam , Amsterdam (Paul Peter Tak, Niek de Vries)
  - Karolinska Institute (Lars Klareskog)
  - Istituto G Galini, University of Genova , paediatric patients (Nicola Ruperto)
- **Intestinal Bowel Diseases: GETAID (Mathieu Allez) (20 French, Belgium centers)**
  - RAMBA Health Care Campus, Haifa (Yehuda Chowers)
  - Chaim Sheba Medical Center (Shomron Ben Horin)
- **Hemophilia: University Clinic, Bonn (Johannes Oldenburg)**
  - Paul-Ehrlich-Institute, Langen (Rainer Seitz)
  - Goethe University, Frankfurt (Wolfhart Kreuz)
- **Multiple Sclerosis: Karolinska Institute, Stockholm (Anna Fogdell-Hahn)**
  - Innsbruck Medical University , Innsbruck (Florian Deisenhammer)
  - Heinrich Heine University, Düsseldorf (Hans-Peter Hartung)
  - Copenhagen University Hopital Rigshospitalelet, Copenhagen (Per Soelberg Sorensen)
  - University Basel Hospital, Basel (Raija Lindberg)
  - General Charles University, Pragua (Eva Havrdova)
  - Hospital Univeritari Vall d'Hebron, Barcelona (Xavier Montalban)
  - Blizard Institute of Cell and Molecular Medecine, London (Gavin Giovannoni)
  - Technischen Universität, München (Bernhard Hemmer)

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Prospective cohort progress

- Scientific protocols have been decided for each diseases with primary objective, secondary objectives, timing for blood collection…
- Next step…
  - to complete the regulatory requirements for clinical trial application in each country
  - Set the sample management including coding and biobanking
- Major prospective cohorts will enrole 200 patients in IBD, 300 patients in RA, MS numbers are still under discussion
- There will be also dedicated samples for « ad-hoc » cohorts: HLA agretopes determination….

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Assay development and validation

- Academic laboratories and SMEs have sent their updated protocols for review by EFPIA « ad-hoc » and independent committees. Goal: to meet industry standards
  - A first round of review has been completed and additional experiments are ongoing
  - Final product and decision June 2013
- Central labs will be selected for dosing samples for each BPs
  - Cut-off values will be set up
  - Common read-out : Results will be given in titers on positive samples
  - Human ADA standards will be produced and used for ADA testing (A. Lanzavecchia)

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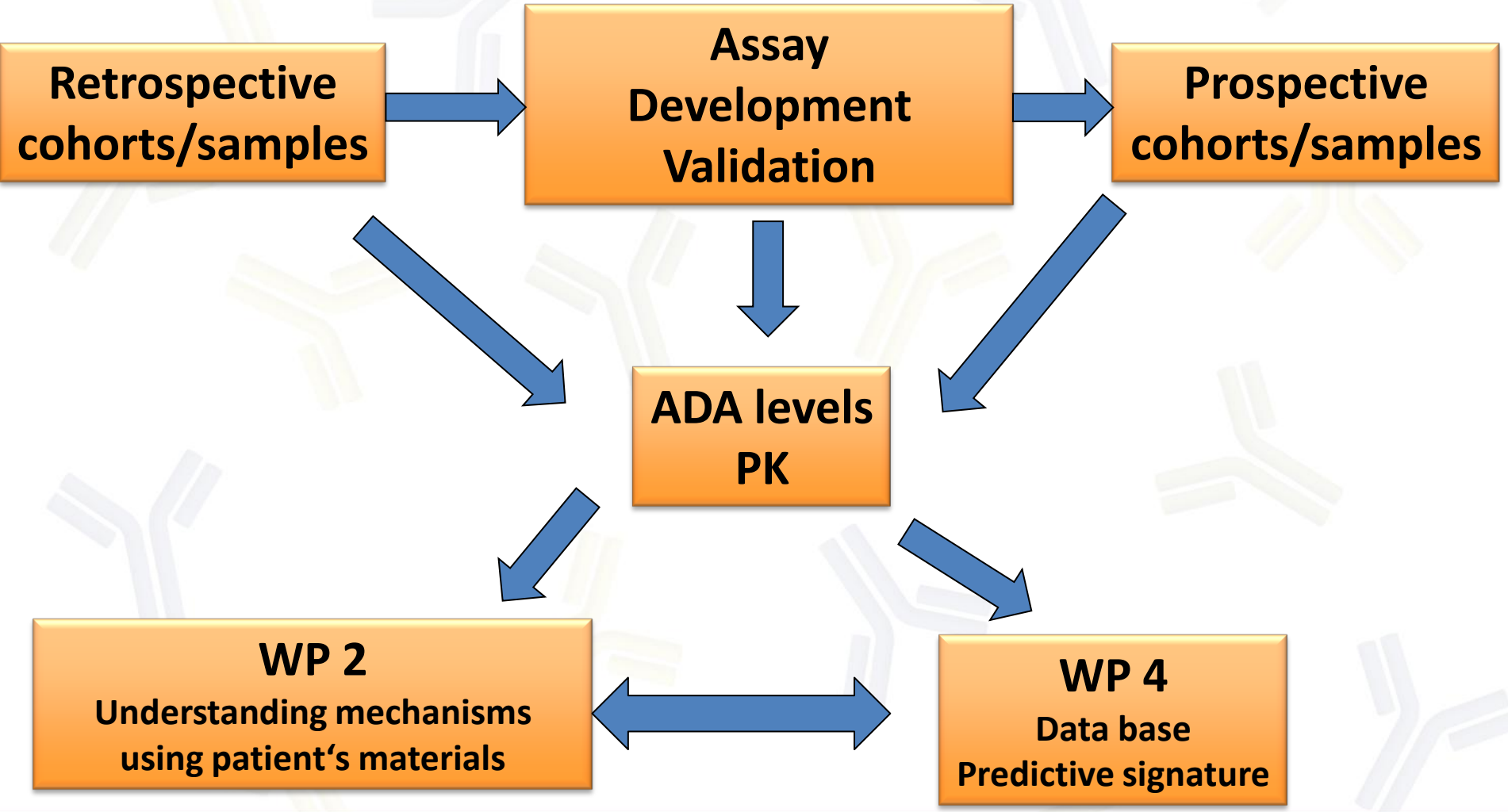
WP 1 organisation



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WP2
Cellular Characterization

- **Ex vivo analysis of PBMC**
  - Human Cell Surface Marker Screening Panel: BD® Lyoplate Technology including 242 phenotypic markers.
- **Evaluation of AD T cell responses**
  - Cytokine profiles (Th1/Th2/Th17) and activation markers *ex vivo* and in response to *in vitro* stimulation with BP or conventional stimuli
  - Regulatory T cell phenotype and function (CD4+ and CD8+, $CD25^{high}$, Helios, CD127-, Foxp3+, IL-10 production)
- **Evaluation of AD B cell responses**
  - Extensive profiling of B cell markers CD19, CD24, CD38, CD1d, IgD, IgM and CD5, CD10
  - T follicular helper cell subsets: CD4+CXCR5+ ICOSL skewed towards a Th1 (CD4+, CXCR5+, CXCR3+, CCR6), Th2 (CD4+, CXCR5+, CXCR3-, CCR6-) or Th17 (CD4+, CXCR3-, CCR6+) phenotype will be assessed.
  - *TFH: plasmablasts ratios will be calculated in ADA+ and ADA- patients*
  - Numerical and functional analysis of regulatory B cells in ADA+/ ADA- patients
- **T- and B-cell AD responses: Clonality analysis and epitope mapping**
  - Next generation sequencing (NGS) to screen T- and B-cell repertoire for clonal expansion

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WP2
Epitope, antibody characterization

- **Identification of in vivo generated BP-derived HLA agretopes**
  - MHC-associated Peptide Proteomics (MAPPs) technology
  - Selected peptides will be used to stimulate T cells from patients
- **Crystal structure determination and epitope analysis**
- **Generation of a repertoire of BP-specific monoclonal ADA**
  - Generate arrays of monoclonal antibodies specific for the different BPs
- **Functional and structural characterization of ADA**
  - Fine epitope specificity of ADA.
  - Avidity of binding of ADA to BP
  - Glycosylation of ADA

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Immunophenotyping

BD Lyoplate™
Human Cell Surface Marker Screening technology
242 antibody and isotype controls

CD1a	CD36	CD66b	CD114	CD165	CD294
CD1b	CD37	CD66f	CD116	CD166	CD305
CD1d	CD38	CD69	CD117	CD171	CD309
CD2	CD39	CD70	CD118	CD172b	CD314
CD3	CD40	CD71	CD119	CD177	CD321
CD4	CD41a	CD72	CD120a	CD178	CD326
CD4v4	CD41b	CD73	CD120b	CD180	CDw327
CD5	CD42a	CD74	CD121a	CD181	CDw328
CD6	CD42b	CD75	CD121b	CD183	CDw329
CD7	CD43	CD77	CD122	CD184	CD335
CD8a	CD44	CD79b	CD123	CD193	CD336
CD8b	CD45	CD80	CD124	CD195	CD337
CD9	CD45RA	CD81	CD126	CD196	CD338
CD10	CD45RB	CD83	CD127	CD197	CD340
CD11a	CD45RO	CD84	CD128b	CD200	α/β TCR
CD11b	CD46	CD85	CD130	CD201	β2-MG
CD11c	CD47	CD86	CD132	CD205	BLTR-1
CD13	CD48	CD87	CD134	CD206	CLIP
CD14	CD49a	CD88	CD135	CD209	CMRF-44
CD15	CD49b	CD89	CD137	CD210	CMRF-56
CD15s	CD49c	CD90	CD138 Ligand	CD212	EGF-R
CD16	CD49d	CD91	CD138	CD220	fMLP-R
CD18	CD49e	CDw93	CD140a	CD221	γ/δ TCR
CD19	CD49f	CD94	CD140b	CD226	Hem. Prog. Cell
CD20	CD50	CD95	CD141	CD227	HLA-A,B,C
CD21	CD51/61	CD97	CD142	CD229	HLA-A2
CD22	CD53	CD98	CD144	CD231	HLA-DQ
CD23	CD54	CD99	CD146	CD235a	HLA-DR
CD24	CD55	CD99R	CD147	CD243	HLA-DR,DP,DQ
CD25	CD56	CD100	CD150	CD244	Invariant NKT
CD26	CD57	CD102	CD151	CD255	Disialogang. GD2
CD27	CD58	CD103	CD152	CD267	MIC A/B
CD28	CD59	CD104	CD153	CD268	NKB1
CD29	CD61	CD105	CD154	CD271	SSEA-1
CD30	CD62E	C106	CD158a	CD273	SSEA-4
CD31	CD62L	CD107a	CD158b	CD274	TRA-1-60
CD32	CD62P	CD107b	CD161	CD275	TRA-1-81
CD33	CD63	CD108	CD162	CD278	Vβ 23
CD34	CD64	CD109	CD163	CD279	Vβ 8
CD35	CD66 (a,c,d,e)	CD112	CD164	CD282	Cut. Ag
					INT β7
					SSEA-3

> 95
Percentage of positive cells
< 5

Baer PC et al, Stem cells (2013)

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WP3 Mechanisms/prediction

Biotherapeutic proteins
(native, aggregated, post-translationally modified)

Sequence identification:
- In silico
- HLA binding affinity
- MAPPs
- CD4+ T cell epitope mapping

T cell responses:
- T cell assays
- huALN

B cell responses:
- huALN
- PBMC assay
- Mouse models

Biomarkers:
- PBMC assay
- DC assay

Intra-subtask comparison

Intra-WP3 comparison

Correlation with:
Ex vivo T cell responses
Ex vivo T cell epitopes
Ex vivo HLA agretopes
Ex vivo biomarkers

WP2

WP1

WP4

Correlation with ADA frequency to different BPs and different indications.

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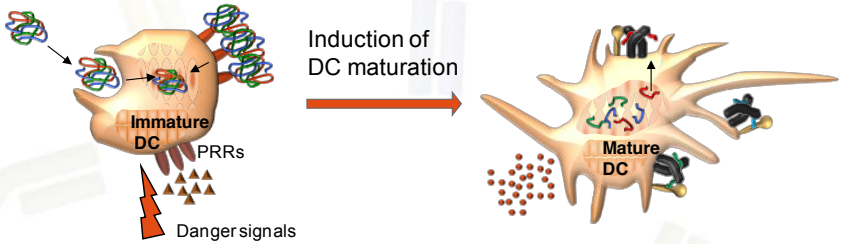
- Dendritic cell activation assay:



Innovative approach to test for unspecific induction of the immune system (aggregates…)

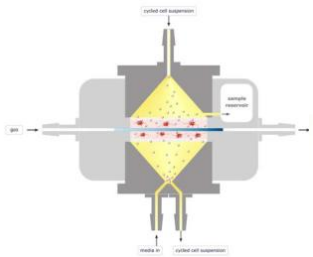
- Humanized mouse models:

Human stem cell transplanted immunodeficient mouse models including double transgenics (HLA-DR1 and human hemophilic factor VIII).

First time use of humanized mouse models to investigate immunogenicity risk of BPs

- Human artificial lymph node:

Human PBMC-based in-vitro system mimicking human lymph node structure.



Only established in-vitro system not only looking at T cell but also B cell activation. First time application of this innovative in-vitro model to BPs.

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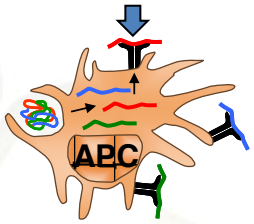
- MAPPs assay



Identification of in vitro generated and presented HLA peptides by human Monocyte-derived Dendritic Cells.

Innovative approach to identify potential T cell epitopes. First time correlation of this technology with other sequence providing technologies.

- Generation of aggregates and PTMs

Generation of well characterized aggregates and PTMs and application in different cell based assay systems.

First time attempt for a comprehensive investigation of the impact of aggregates and PTMs on antigen presentation.

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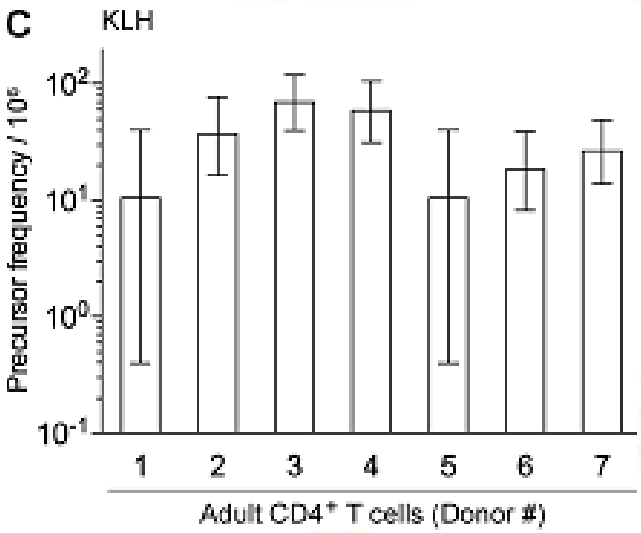
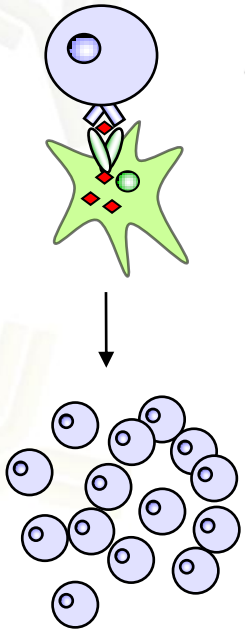
Evaluation of the size of BP-specific T cell repertoire in healthy donors

Rationale: size of the antigen-specific CD4 T cell repertoire shapes the T cell response in vivo (Moon et al , Immunity, 2007; Jenkins et Moon, J Immunol, 2012)

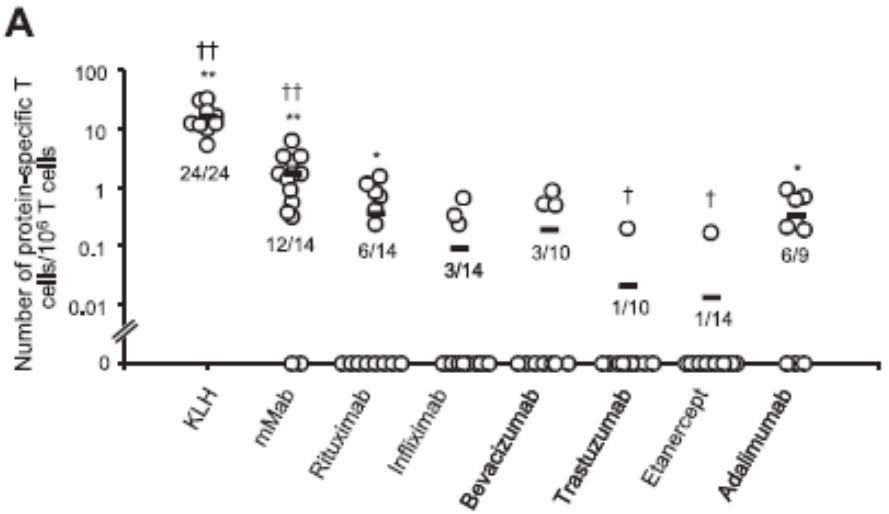
Two independent approaches:

Polyclonal amplification of CD4 T cells
(Geiger et al, JEM, 2009)





Antigen-specific amplification
(Delluc et al, FASEB J, 2011)



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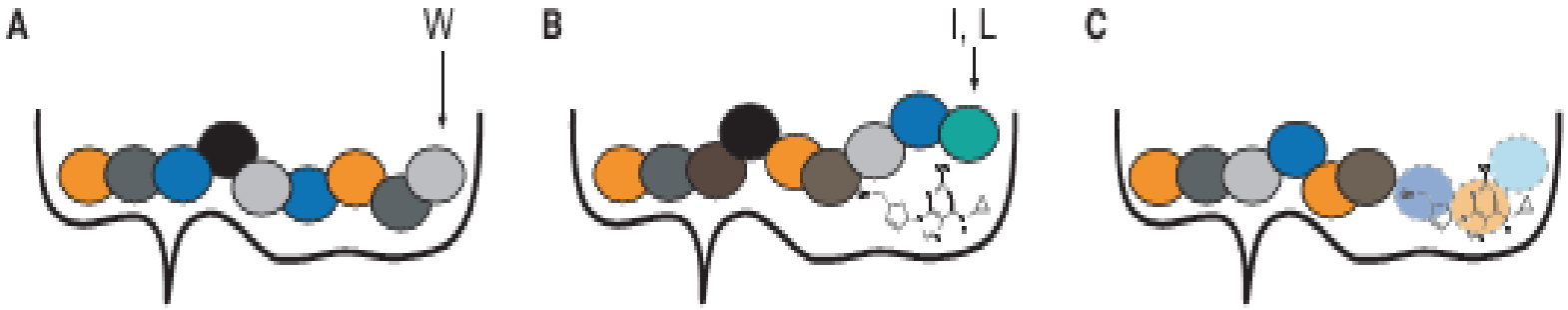
Peptide identification



HLA-B*57:01 + 'normal' self-peptide repertoire

HLA-B*57:01 + abacavir + altered self-peptide repertoire

HLA-B*57:01 + abacavir + 'normal' or altered self-peptide

Yun J et al, Allergy (2012)

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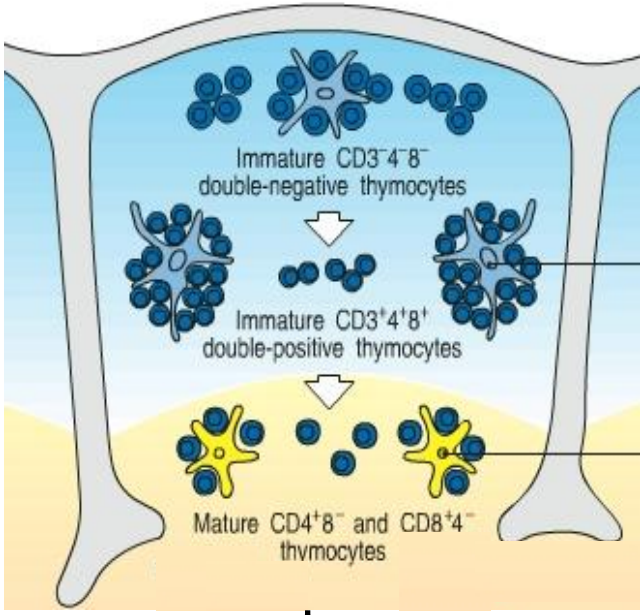
hCD34+ stem cell

Thymus

NOG mouse® ; NSG mice
Rag2°IL2rcγ° mice

HLA-A2/DR1/IAβ°/β2m°
Rag2°IL2rcγ° mice



epithelial cells
positive selection

dendritic cells
negative selection

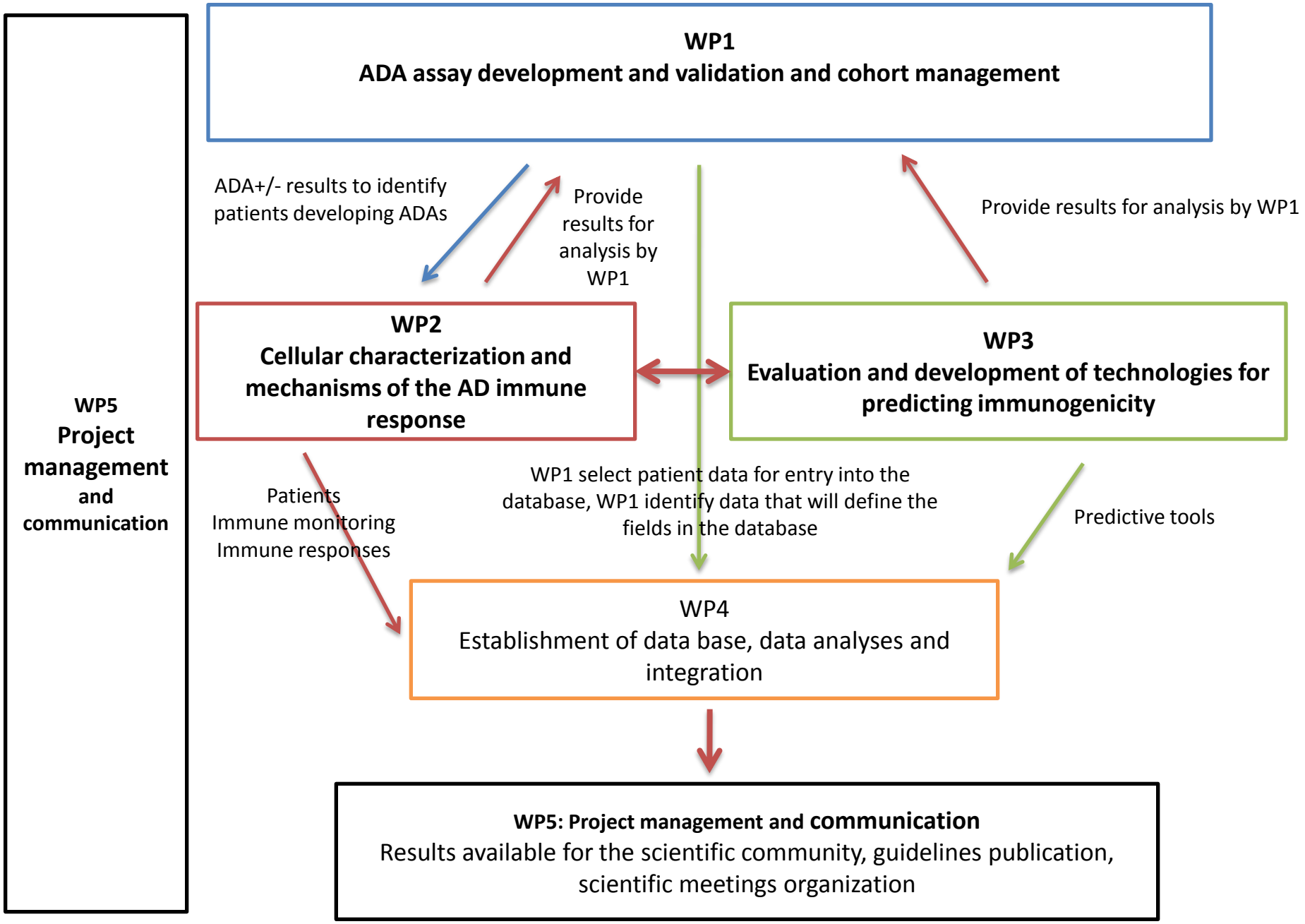
hu-macrophages
hu-dendritic cells
hu-CD4Lymphocytes **H2**-restricted
hu-CD8Lymphocytes **H2**-restricted
Immature B cells (IgM, IgG) ✗

Reconstitution of human immune system cells of human origin

hu-macrophages
hu-dentitic cells
hu-CD4Lymphocytes **HLA**-restricted
hu-CD8Lymphocytes **HLA**-restricted
Immature B cells (IgM, IgG)

- Responses : T epitope H2-restricted
- T cells no tolerance to HLA-expressed cells
- **No correlation with human clinical results**

- Responses : T epitope HLA-restricted
- T cells tolerant to HLA-expressed cells
- Correlation with human clinical results


WP1
ADA assay development and validation and cohort management
ADA+/- results to identify patients developing ADAs
Provide results for analysis by WP1
Provide results for analysis by WP1
WP2
Cellular characterization and mechanisms of the AD immune response
WP3
Evaluation and development of technologies for predicting immunogenicity
WP5
Project management and communication
Patients
Immune monitoring
Immune responses
WP1 select patient data for entry into the database, WP1 identify data that will define the fields in the database
Predictive tools
WP4
Establishment of data base, data analyses and integration
WP5: Project management and communication
Results available for the scientific community, guidelines publication, scientific meetings organization

ABIRISK management

- **ABIRISK documents for internal use**
  - Immunogenicity “Terms and definitions” document
  - Data policy and integrity document
  - Publication policy document
  - Sample management and bio-banking document in progress
  - Bi-monthly internal newsletter
- **Day to day management**
  - Executive Management Team bi-monthly TC meeting (WP leaders, Cohort leaders, managers)
  - WP TCs once a month with the managers
  - Steering Committee once a year (Next in Basel, September 2013)
  - General Assembly once a year (next one in Siena, March 2013) with the Scientific Advisory Board
    - Roland Liblau (INSERM, France), Amy Rosenberg (Division therapeutic proteins, FDA), Christian Schneider (EMA, CAT Chairman, DKMA), Robin Thorpe (NIBSC, UK), Severine Vermeire (Division of Gastroenterology, University Hospital Leuven)

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ABIRISK missions

- Be an unique place providing information on BP immunogenicity
  - External Newsletter for identified stakeholders
  - Monthly Scientific Newsletter
  - Website www.abirisk.eu
  - LinkedIn discussion group

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ABIRISK regulatory relevance

- Immunogenicity as it relates to efficacy and safaty of BPs is a rapidly evolving field of study
- Diseases requiring the use of BPs are complex and severe in nature, and the drug development process can be extensive
- Elucidation of the underlying mechanisms of immunogenicity may result
  - in more science-based regulatory guidelines, which may reduce the immunogenicity testing burden
  - In saving time and resources in the BP drug development process
  - In enabling new medicines to reach patients

Dan Sikkema, IMI EMA meeting, London 2012

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