



Innovative Medicines Initiative

ABIRISK

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

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EIP Meeting, Copenhagen February 8th 2012

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

Objectives and driving forces (2)



- Complementary expertise for ADA assays
 - Standardization and characterization of ADA
 - SOP for each assay
 - Universal assay validation protocol
 - Generation of an internal standard
- Novel approaches to characterize AD lymphocyte responses
 - Retrospective and prospective patient samples
- Development and validation of innovative prediction tools
- Collection and integration of immunogenicity-related data and clinical relevance of ADA
 - Predictive signatures for immunogenicity phenotypes and immunogenicity-related clinical events

WPs



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- WP1 “ADA assay development and validation and cohort management”
 - WP2 “Cellular characterization and mechanisms of the AD immune response”
 - WP3 “Evaluation and development of technologies for predicting immunogenicity”
 - WP4 “Establishment of a data base, data analyses and integration”
 - WP5 “Project management and communication”



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WP 1

“ADA assay development and validation and cohort management”



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WP1, Task 1 and related objectives



Task WP1.1

Standardization of definitions and terminology related to immunogenicity, its prediction and associated clinical events.



Objective

To provide clear definitions around terms and concepts related to immunogenicity, its prediction and associated clinical events (e.g., transient ADA response, pre-existing ADA, assay sensitivity, loss of response, allergic reaction).

Achieved by workshop at Month 2



Milestone

Report of workshop on standardized ADA assay development and validation for each selected biopharm product. Report will also include a list of definitions and terms related to immunogenicity, its prediction and clinical effects.

WP1, Task 2 and related objectives



Task WP1.2

Development and Validation of Standardized Anti-Drug Antibody (ADA) and Neutralizing Antibody (NAb) Assays



Objective

- To provide and develop validated assays for detecting biopharmaceutical-associated immunogenicity. **To produce standards for ADA quantification**
- To define criteria characterizing ADA+ and ADA- patients for each selected biological product

Using guidelines for assay validation

Shankar et al. 2008, J. Pharm. Biomed Anal. 48:1267-1281

EMA Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins (April 2008)

FDA DRAFT guidance on Assay Development for Immunogenicity Testing of Therapeutic Proteins (Dec 2009)



M15

- Standard ADA assay protocol distributed for validated assays
- Assay cut-off values determined

Task 2, Number of patients available



N patients in retrospective cohorts by treatment

Rx	Serum	DNA	RNA	Cells	% ADA+/NAb+ patients
IFN-beta	2200	4500	3300	0	40-80/5-25
Natalizumab	3000	1000	735	0	9/5
Infliximab	1430	700	0	0	45/?
Adalimumab	500	820	30	20	20-30/?
Etanercept	260	26	0	0	2%/?
Rituximab	265	26	0	0	?/?
Factor VIII	1000	150	1	5	5-30/5-30

Rx	<u>Minimum</u> # of patients available for validation
IFN-beta	110
Natalizumab	150
Infliximab	250
Adalimumab	50
Etanercept	5
Rituximab	?
Factor VIII	50

- Partial validation needed for IFNbeta, NTZ, FVIII
- Full validation for anti-TNFalpha
- New assays developed for RTX
- Validation report for all treatment groups will be provided
- Essential for patient stratification

Task 3 and related objectives



Cohorts management



Objectives

- To identify the type of data that will define the fields in the database (i.e. related to product characteristics, patient-related information, immunogenicity data, clinical endpoints)
- To provide and evaluate patient data for entry into the database and establish patient cohorts to be included in the ABIRISK project (retrospective and prospective studies)
- To evaluate and select data generated using various predictive immunogenicity tools and assays, such as patient's HLA status, T cell epitope content, T cell activation response, for entry into the database.

Task 3....



Retrospective cohorts

Serum and genetic material available →
ADA/NAB assay development and
validation, genetic factors

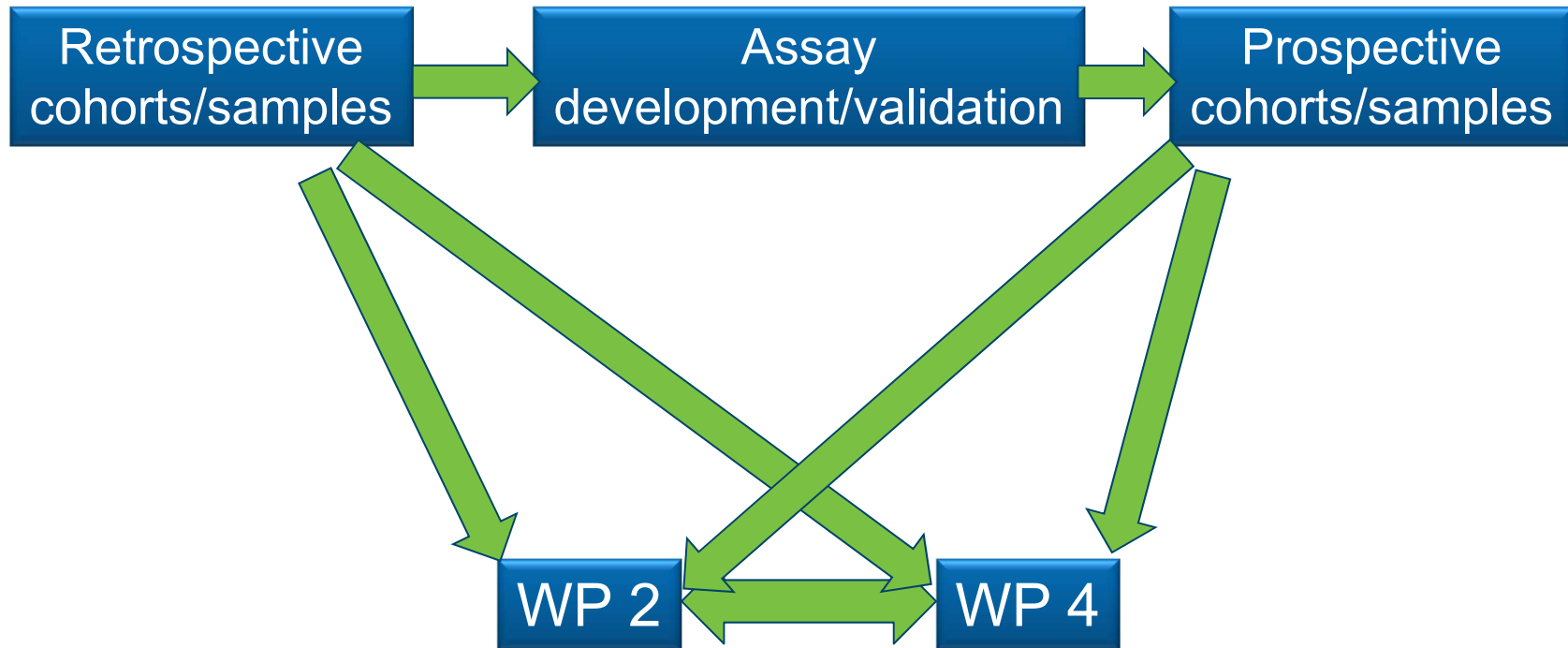
Prospective cohorts

All types of sample material →
Experimental work WP2
Define outcomes

Rx	Serum	DNA	RNA	Cells	% ADA+/NAb+ patients
IFN-beta	1185	485	365	260	40-80/5-25
Natalizumab	970	570	500	335	9/5
Infliximab	230 (+400 PRINTO?)	230	230	210	45/?
Adalimumab	330 (+400 PRINTO?)	330	330	330	20-30/?
Etanercept	130	130	130	130	2%/?
Rituximab	150	150	150	150	?/?
Factor VIII	130	130	15	35	5-30/5-30

N patients in prospective
cohorts by treatment

WP 1 organisation





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WP 2

“Cellular characterization and mechanisms of the AD immune response”

Aim: understanding mechanisms using patient’s materials



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- ***Patients and controls:***
 - SLE patients treated with rituximab;
 - IBD and RA patients treated with TNF α agonists
 - HA patients treated with FVIII
 - MS treated with IFN β (3 different types)/natalizumab
 - Controls: Healthy donors and patients not treated with BP
 - Longitudinal samples from patients before and after BP treatment
 - ADA+ and ADA- patients will be assessed
 - **Retrospective samples are available and prospective samples will be collected.**
 - ***Preliminary work:***
 - Validate retrospective sample cell viability for use in experiments
 - Standardize methodology for prospective sample collection and exchange between partners in the consortia.
 - Train consortia partners to ensure standardization of sample collection and storage. On site visits
 - **Local Ethical Committee approval is in place, or has been applied for**
-

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

Epitope and 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
 - NGS technology to compare in vitro T-cell responses to specific peptides with in vivo responses to intact biologicals in 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
 - Affinity of binding of IgG ADA to FVIII
 - Glycosylation of ADA
- **Kinetic development of binding antibodies against IFN for the prediction of neutralizing antibodies**

Genetic markers of BP immunogenicity

- To identify genetic markers predisposing to BP immunogenicity
 - Characterization of genetic factors contributing to ADA development in MS, IBD, RA and HA
 - Collaboration with R. Plenge (Boston), anti-TNF and RA; 4 ABIRISK cohorts are included (EAC, PHARE, AMC, EIRA)



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WP 3

“Evaluation and development of technologies for predicting immunogenicity”



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WP-3 Predicting Immunogenicity



- Balanced mix of expert groups from academia, SMEs and EFPIA companies:
 - 7 groups from EFPIA
 - 7 groups from academia/SME
- The main goals of the of work package are
 - To evaluate clinical relevance and gain a greater understanding of technologies and companies with respect to prediction of immunogenicity;
 - To develop and assess novel prediction methods;
 - To assess effects of formulation and aggregation on immunogenicity;
 - To provide appropriate deliverables compatible with the integration in the ABIRISK database and comparison with data obtained from WP1 and WP2 as part of WP4
 - Evaluation of clinical relevance of the prediction methods will be achieved by applying the relevant in-vitro methods of WP3 also on ex-vivo samples in WP2. A direct comparison between the obtained results performed as part of WP4, will allow clinical validation of the respective methods.

Work Package 3 overview

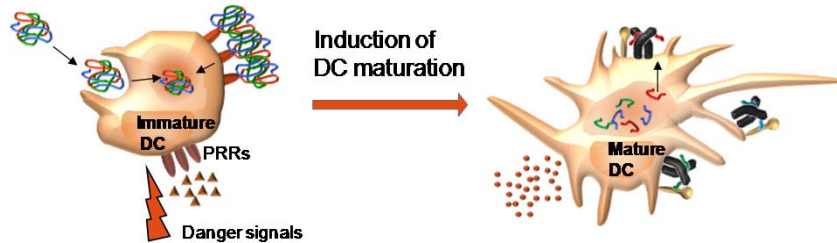


Objectives	Work package sub-tasks addressing the aims
Evaluate clinical relevance and gain a greater understanding of technologies and companies with respect to prediction of immunogenicity.	<ul style="list-style-type: none">✓ Evaluation of different T cell assay approaches (WP 3.1)✓ Evaluation of different in silico prediction methods (WP 3.2)✓ Identification naturally processed HLA peptides by MAPPs (WP 3.3)✓ Mapping of CD4+ T-cell epitopes (WP 3.4)✓ Peptide affinity for HLA class II (WP 3.5)
Develop and assess novel prediction methods.	<ul style="list-style-type: none">✓ In vitro modulation of dendritic cell function and activation by BP (WP 3.6)✓ Development of innovative in vitro PBMC assay (WP 3.7)✓ Evaluation of the Artificial Lymph Node system (WP 3.8)✓ Relevance of innovative animal models (WP 3.9)
Assess effects of formulation and aggregation on immunogenicity.	<ul style="list-style-type: none">✓ Generation of post-translational modifications and aggregates and their characterization (WP 3.10)✓ Test modified BPs for their effect in established and newly developed prediction models (WP3.5, WP3.6, WP3.7 and WP3.9).

Details to innovative prediction methods



- Dendritic cell activation assay:



Innovative approach to test for unspecific induction of the immune system.

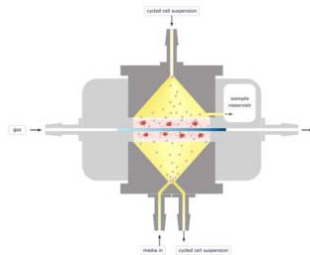
- Humanized mouse models:



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, especially in HLA-DR1 and Hemophilic factor VIII transgenic mouse models.

- Human artificial lymph node:



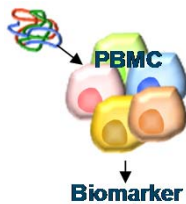
Human PBMC-based in-vitro system mimicking human lymph node structure.

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.

Details to innovative prediction methods



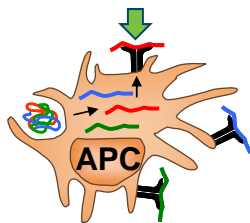
- PBMC assay



Development of an innovative cell system based on whole PBMC cultures to be as close to in vivo situation as possible.

Innovative approach to identify (new) biomarkers for immunogenicity prediction and patient stratification.

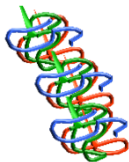
- MAPPs assay



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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WP 4

“Establishment of a data base, data analyses and integration”



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WP4: Establishment of database, data analyses and data integration



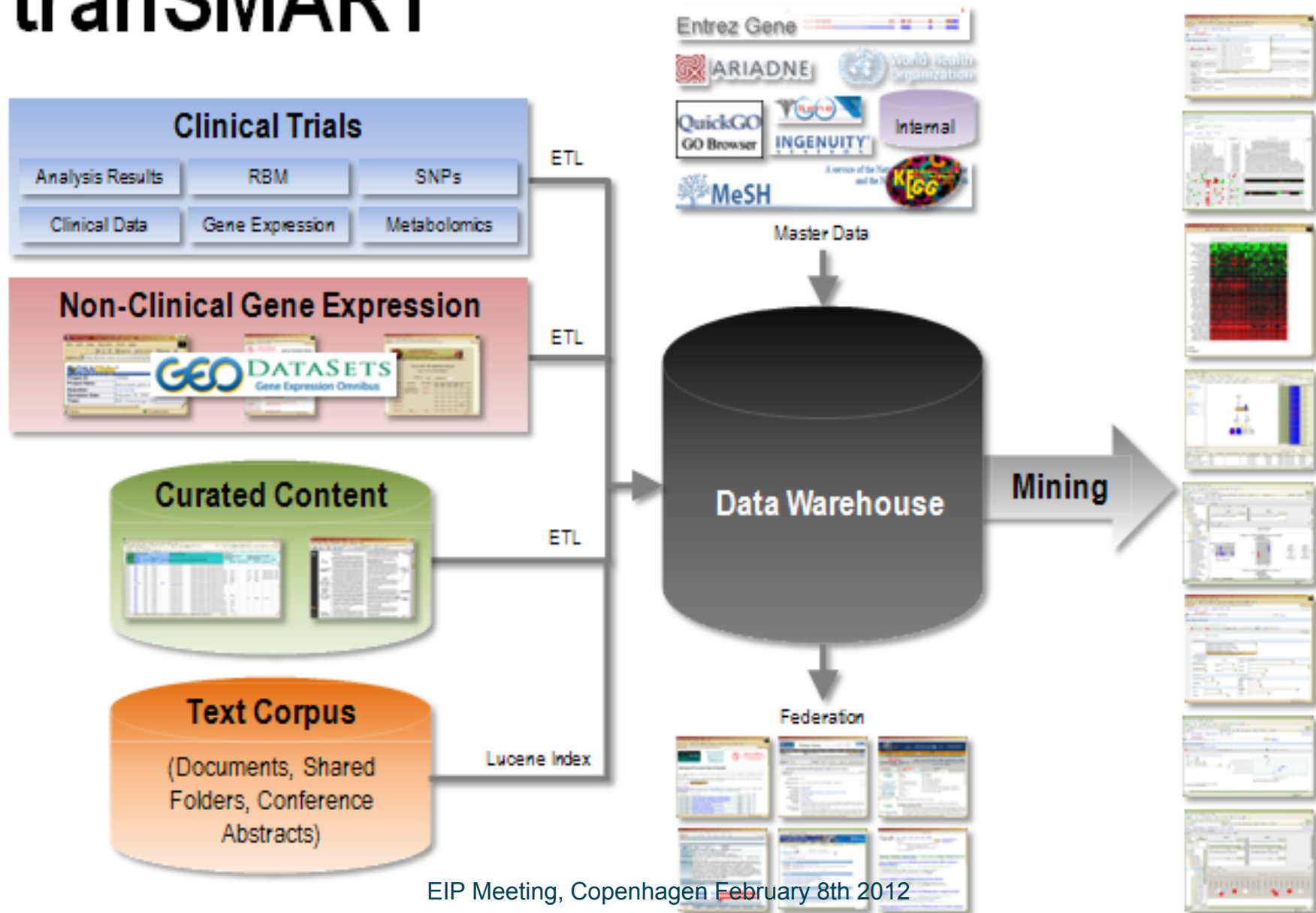
- Objectives
 - Part A: Design and maintain database
 - Housing preclinical and clinical data
 - Data feasibility assessment in collaboration with WP1
 - Design common data model for existing and future data
 - Leverage the tranSMART datawarehouse system
 - Through eTRIKS (IMI 4 proposal) designed to harmonize processes and further reduce costs for IMI projects
 - **Timing of availability of eTRIKS coincides with timing of data entry in ABIRISK**
 - Data will be deidentified, and legal consents obtained
 - Controlled access for current and future research questions

tranSMART



- Developed by Johnson and Johnson
- Fully translational data warehouse based on open-source informatics platform (NIH and Harvard Med School)
- Cost reductions through hosting on Amazon Elastic Compute Cloud
- Adapted by IMI as an open-source knowledge management platform
 - Current projects: Ubiopred, OncoTrack, Safe-T
- Capability to house
 - Clinical data, clinical and pre-clinical gene expression, protein profiling (ELISA, RBM), SNP, PD markers, metabolomics, proteomics
 - In-house – immunology (large and small mol), oncology, cardiovascular, psychiatry
 - Public and commercial
- Data
 - Curated text & Text indexing
 - Master data, ontologies, vocabularies and metadata
 - Federated sources
- Strict security policies, fully auditable

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eTRIKS mission

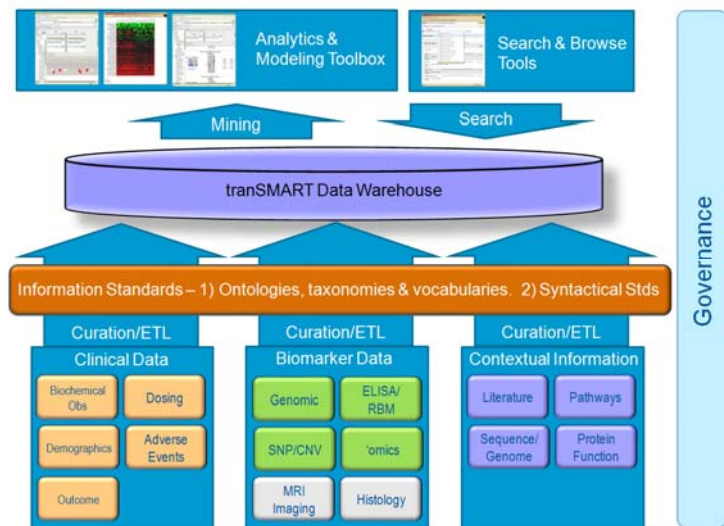


To provide a secure, open-source, low-cost and high quality technology infrastructure for industry, academics, government and NGOs to share data and conduct advanced translational knowledge management internally and collaboratively via a hosted framework.

eTRIKS: IMI Call 4



- **Problem:** No open KM infrastructure to support pre-competitive (& competitive) cross-institute TR. No public knowledge base of curated TR data.



- **Consortia Model & Costs:**

- 5 year funding model
- Core group of ~12 individuals
- 3-5 organisations with experience in TR KM service delivery
- Review impact in year 2: expect need to increase funding as trial number & data types supported increases
- could develop into 'Big Idea'

- **Proposal:**

- Consortia to develop & deliver an open TR KM infrastructure & Service
- Built around **J&J's tranSMART** open platform
- Curation of content (live and historic TR trials)
- Support of existing IMI initiatives
 - *UBIOPRED, OncoTrack, Safe-T, etc...*
- TR KM standards, enabling TR data sharing
- Promotion of TR analytics innovation.
- Mirror of US tranSMART consortia (tba)

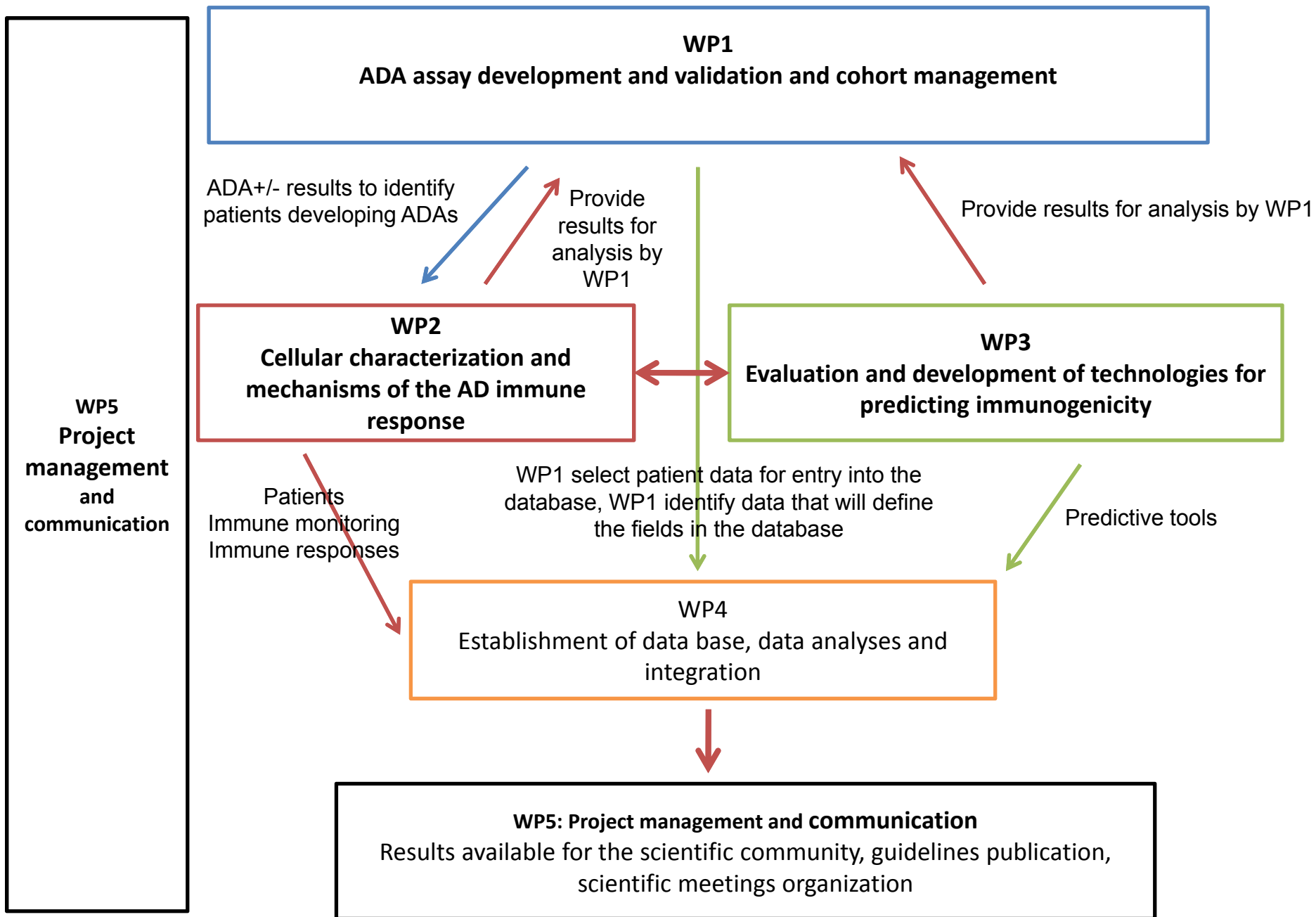
- **Benefits:**

- Improved TR project operating efficiencies
- Stable legacy: IMI TR data security
- Access to curated historical content enabling both:
 - Analytics/Methodology innovation
 - Novel x-study translational discovery
- Enables x-institute TR data sharing
- Strengthened TR Ix community

WP4: Establishment of database, data analyses and data integration



- Objectives (cont)
 - Part B (5 subtasks):
 - Define protocols for the validation and standardization of assays for identification of ADA (with WP1)
 - Evaluate assays for classification of types of ADA
 - Correlate prediction methods (WP3) with clinical ADA incidence (WP1) and ex vivo generated cellular responses (WP2)
 - Derive biostatistical predictive signatures of immunogenicity phenotypes and outcomes (LOR, safety)
 - Describe natural history of ADA development
 - Identify variables associated with ADA and ADA related outcomes
 - Develop statistical models specific to development of immunogenicity in patients with hemophilia : tool for pharmacovigilance (to be extended to other BPs)



ABIRISK impact on drug development



- New and better predictive tools are needed to assess candidates prior to FTIH studies
- The ability to select the best candidate from multiple clones may allow companies to provide medicines that will benefit larger populations of individuals for longer periods of time
- Complex BP molecules are in development (multi-specific, functional enhancement (ADCC, CDC, etc), antibody fragments, and many more):
 - The ability to assess their immunogenicity potential in vitro will be key to successfully launching these new medicines and maximising their therapeutic potential
 - Patient stratification and early identification of clinically relevant immunogenicity will also be a key feature of this project
 - Assessment of formulations and aggregates and their effect on immunogenicity will guide future drug development

Conclusions



- ABIRISK is an unique consortium of academic and EFPIA partners possessing a broad diversity of complementary competences
- ABIRISK have access to large cohorts of patients treated with BPs allowing the potential identification of common threads across treatments and diseases
- ABIRISK has the goal to create an unique data base allowing the collection of numerous informations on BPs immunogenicity and its consequences
- We believe that this could be enormously helpful in future drug design in the field of biotherapy