CIP
CIMT Immunoguiding Program

30th September 2010,
Washington

"Harmonization of Immunological Monitoring Across Institutions"

C.M. Britten

Disclosure Information

CEDRIK M. BRITTEN

The following relationships exist related to this presentation:

50% Employee (University Medical Center of the Johannes Gutenberg-University, Mainz, Germany)

50% Employee (BioNTech AG, Mainz, Germany)

Today‘s Panel Discussion

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9:15 am-9:45 am Panel Discussion

Panelists: Session 1 Speakers; Sylvia Janetzki and Michael Kalos

- How can assays be “harmonized” across institutions ?
- What should be included in all publications which include immunological monitoring data?

CIP - Activities


C.
Gouttefangeas


C.
Britten


S.
Van der Burg
(Current Chair)


C.
Ottensmeier


M.
Welters


M.
Kalos

Scientific Sessions + Workshops

Proficiency Panel Program

Co-operation with other groups

Research

Publications

HLA-peptide Multimers

ELISPOT

Intracellular Cytokine Staining

Proficiency Panel Programs

In 2005 two independent harmonization initiatives have launched their proficiency panel programs

CIMT Immunoguiding Program („CIP“)
as part of the Association for Immunotherapy of Cancer
(www.cimt.eu)

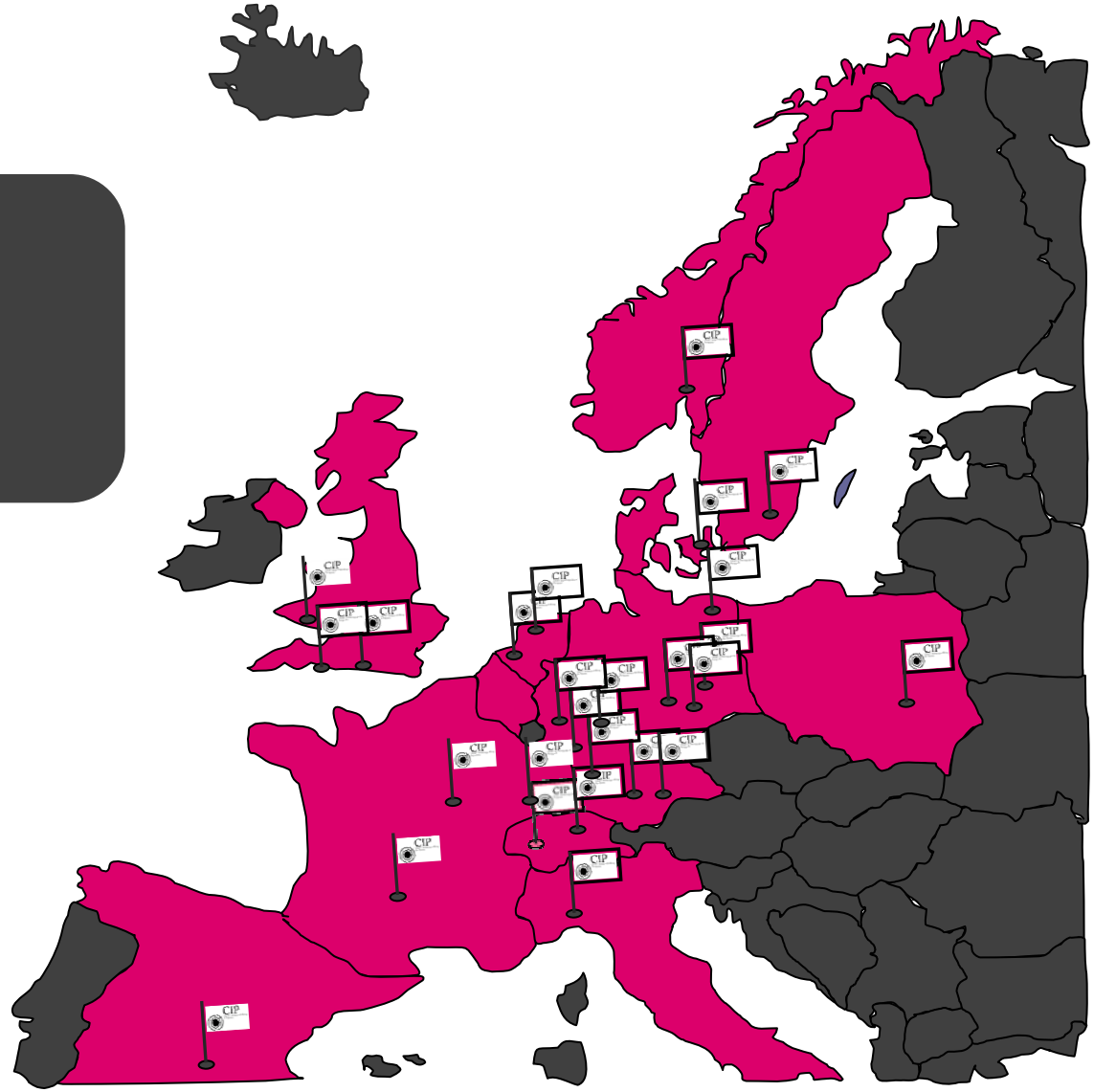
CIC Proficiency Panel Program
as part of the Cancer Research Institute
(http://www.cancerresearch.org/CancerVaccineConsortium.html)

CIP – Proficiency Panel Programm

CIP
CMT Immunoguiding Program

Participants

- 40 participating labs
- 12 European countries



CIMT – Recent/New panels

CIP_ID07_2009_MUL *(experiments completed)*

Assay(s): HLA-Peptide Multimers
Organizers: C. Gouttefangeas, K. Laske, S. Heidu (Tuebingen), S. Hardrup (Herlev)

CIP_ID08_2010_GAT *(experiments completed)*

Assay(s): Gating Panel (ICS Data)
Organizers: M. J. P. Schoenmaekers-Welters , S.H. van der Burg (Leiden)

CIP_ID09_2010_ELI *(recruiting completed)*

Assay(s): IFN- γ ELISPOT – Media used for thawing and freezing
Organizers: H. Filbert, S. Attig, C. Britten (Mainz)

CIP_ID010_2010_ELI *(recruiting will begin Q3/2010)*

Assay(s): IFN- γ ELISPOT for in vitro stimulated T cells
Organizers: L. low, A. Mander, C. Ottensmeier (Southampton)

CIP_ID011_2010_MUL *(recruiting will begin Q3/2010)*

Assay(s): HLA-Peptide Mutimers – Reference Samples
Organizers: S. Singh and S.H. van der Burg (Leiden)

CIC – Proficiency Panel Program

2005: 1st Elispot panel (36 labs)
2006: 2nd Elispot panel (29 labs)
2007: 3rd Elispot panel (35 labs)
1st Multimer panel (29 labs)
1st ICS panel (28 labs)
1st CFSE panel (21 labs)
2009: 4th Elispot panel (41 labs)
Serum task force
2nd Multimer panel (20 labs)
2nd ICS panel (31 labs)

2010: 1st ICS **Gating** Panel (in wide collaboration)
1st Luminex Panel (in collaboration)
5th Elispot and 3rd Multimer Panel

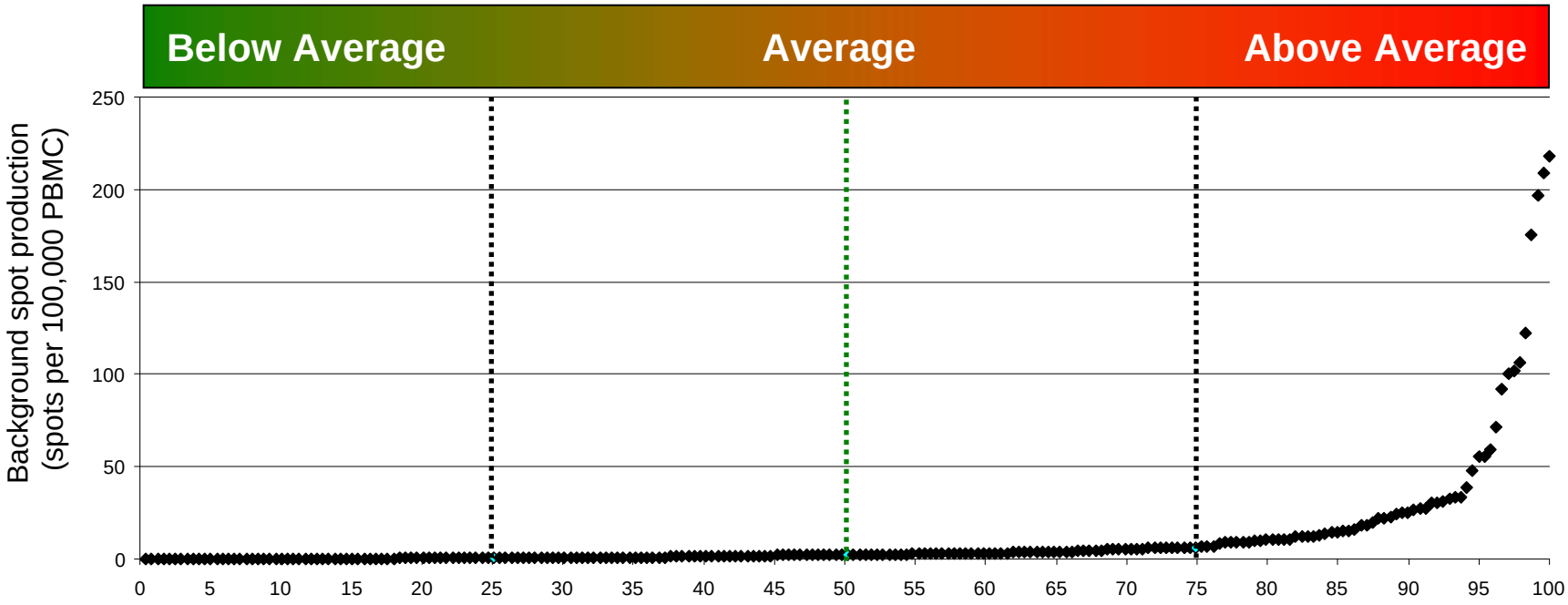
Aims of Proficiency Panel Programs

„Quality Assurance (QA)“
Provide immediate feed-back about performance relative to the group (or to a dynamic reference value)

„Assay Harmonization“
Use the collected data to systematically investigate the performance of subgroups and deduce harmonization guidelines.

„Protocol Optimization”
Use the collected data to systematically identify critical process steps (stimulus for MIATA).

QA: Background spot production expected from a virtual lab with average test performance



Percentile	Classified as	Spots
95th	Extremely high	55.40
75th	Upper end of average	6.33
50th	**Average**	**2.17**
25th	Lower end of average	0.6
5th	Extremely low	0.07

Average:

0.6 - 6 spots per 100,000 PBMC

Median BG:

2 spots per 100,000 PBMC

Harmonization - Response Determination

	Overall (no filters)	
Response Determination Method	Response Detection Rate N=282	False Positive Rate N=196
S > 2-fold BG	74%	17%
S > 2-fold BG and > 5/100,000	59%	3%
S > 2-fold BG and > 10/100,000	49%	1%
S > 3-fold BG	66%	9%
S > 3-fold BF and > 5/100,000	54%	1%
S > 3-fold BG and > 10/100,000	45%	0%
S > 4-fold BG	59%	7%
S > 4-fold and > 5/100,000	49%	1%
S > 4-fold and > 10/100,000	42%	0%
S ≥ 4-fold and ≥ 5/100,000	49%	1%
T-test	76%	10%
DFR(eq)	75%	11%
DFR(2x)	61%	2%

Harmonization is needed to increase comparability of results generated accross insititutions

Moodie et al. 2010

Harmonization – ELISPOT Recommendations CIP

- Refrain from using allogeneic APCs
- Use triplicate wells for each antigen
- Introduce a resting time of the PBMCs before they are added to the ELISPOT plate
- Add an optimal cell number per well *(≥ 4 x 10^5 lymphocytes per well recommended by CIP labs)*
- Use serum-free test conditions
- Use a scientifically sound method for response determination (DFR method proposed by Moodie 2010)

CIC - Initial Elispot Harmonization Guidelines

A. Establish lab Elispot SOP for:

A1. Counting method for apoptotic cells in order to determine adequate cell dilution for plating

A2. Overnight resting of cells prior to plating

B. Use only pretested serum with optimal signal:noise ratio

C. Establish SOP for plate reading, including:

C1. Human auditing during reading process

C2. Adequate adjustment for technical artifacts

D. Only let well trained personnel conduct assay

Janetzki et al., CII. 2008 Mar;57(3):303-15

CIC Cancer Immunotherapy Consortium

Conclusions 1/2

Large-scale Harmonization activities can lead to

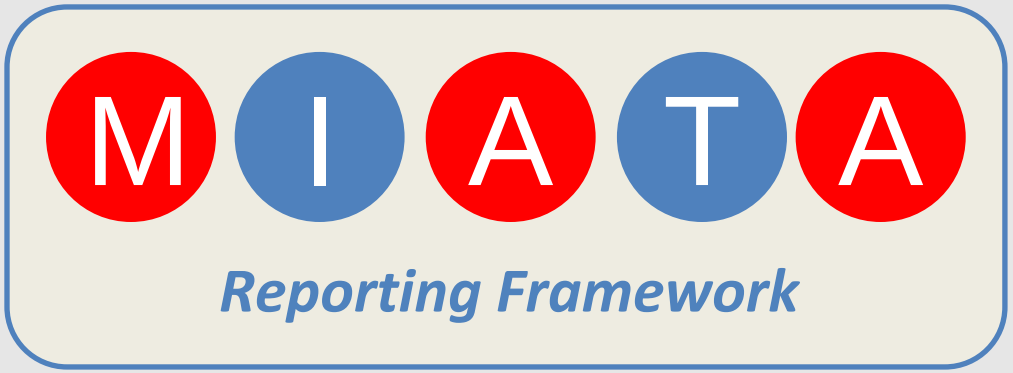
- dynamic **reference values** to rank test performance,
- **increased comparability** of results generated across institutions,
- **improved assay** performance in a group,

...and thus accelerate clinical development of new cancer immunotherapies.

Beginners and **highly experienced** labs can benefit from harmonization efforts.

Minimal Information About T cell Assays (MIATA)

Announced at CIMT2009 and in Immunity in 2009



www.miataproject.org

Cancer Immunotherapy Consortium

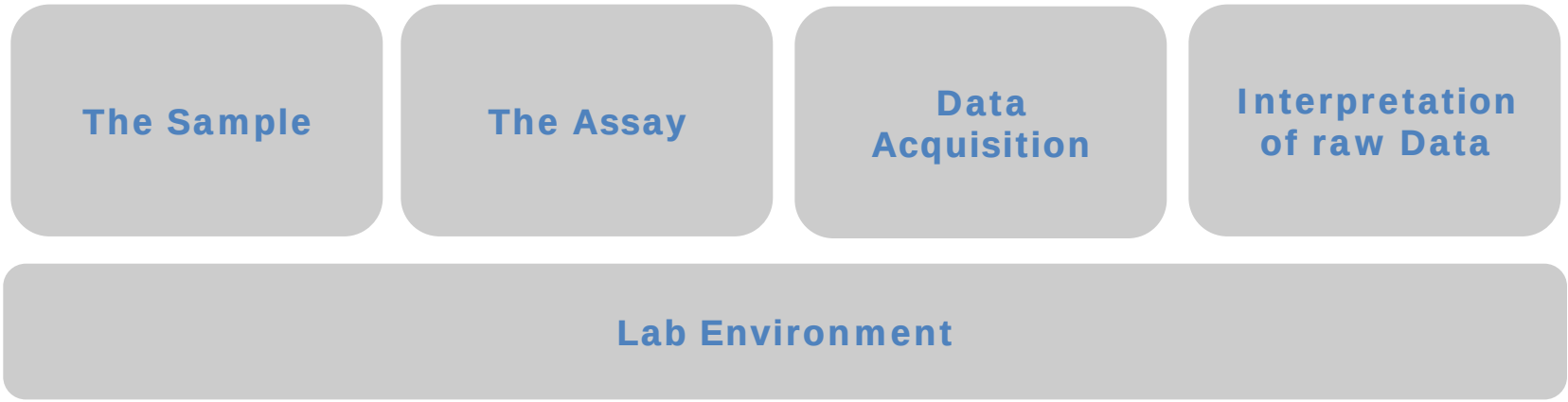
CIP CIMT Immunoguiding Program

NIBIT Network Italiano per la Bioterapia dei Tumori

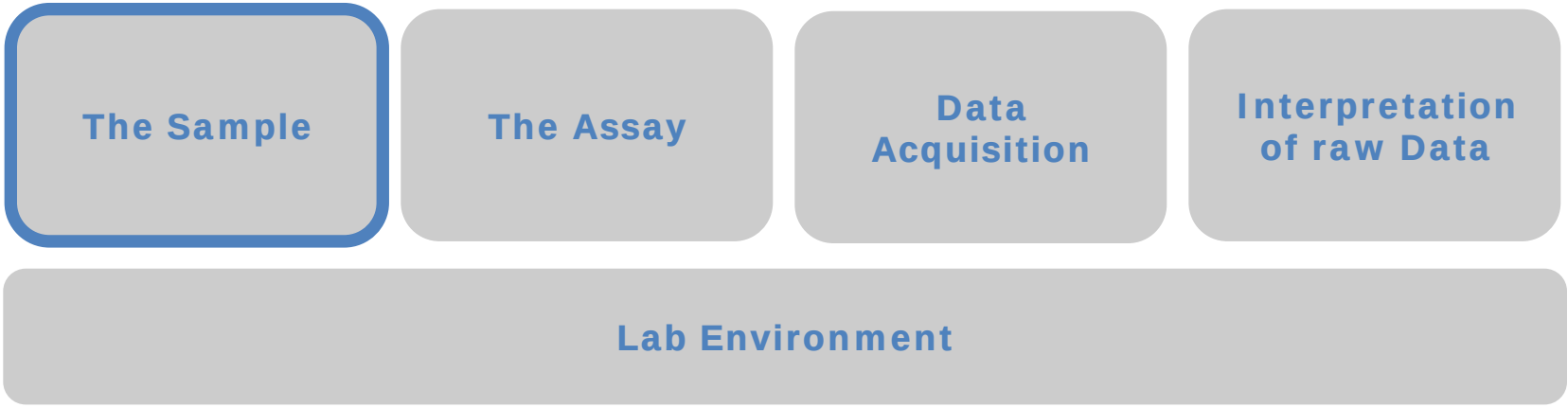
The Human Immune Monitoring Center at Stanford
Immunology for the People!

"MIATA"-minimal information about T cell assays."
Janetzki S, Britten CM, Kalos M, Levitsky HI, Maecker HT, Melief CJ, Old LJ, Romero P, Hoos A, Davis MM. Immunity. 2009 Oct 16;31(4):527-8.

MIATA – 5 Modules



MIATA – 5 Modules



***The Sample - 1D: Quality of cell material**:*

Required:

mean recovery and viability of cell material
method used to determine the cell recovery and viability

Optional:

An expanded list of details on the quality control of the cell material that was tested (of great interest would be (i) specific cut-offs for recovery and viability (if applicable), (ii) how material was treated that did not reach the cut-off and (iii) the mean time laps at which viability was tested relative to the time of thawing and the experiment).

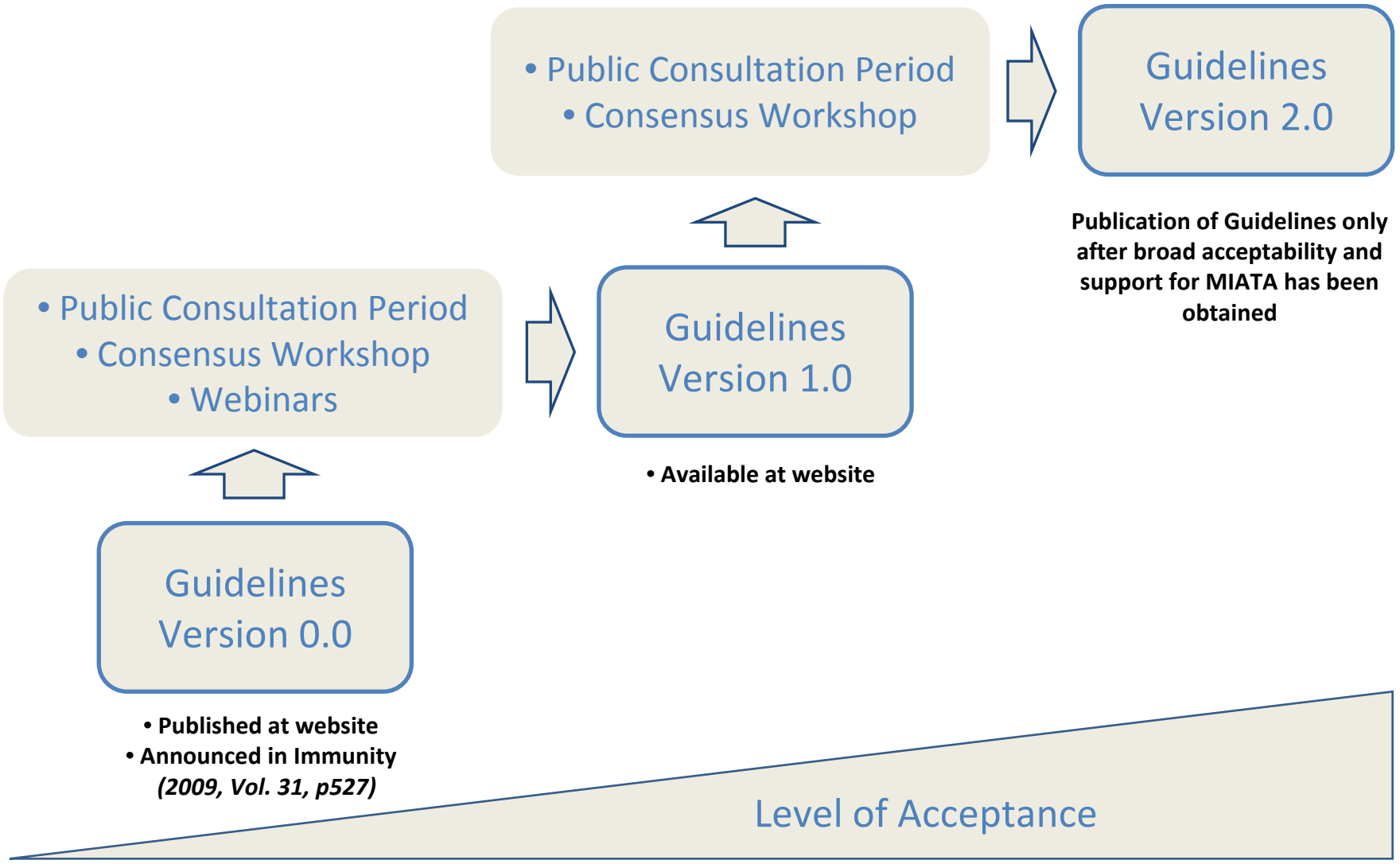
MIATA – Consensus Workshop in Washington

Facilitator:
A. Hoos

Support:

Moderators:
Module 1: C. Britten
Module 2: S. Janetzki
Module 3: K. Odunsi
Module 4: S. van der Burg
Module 5: M. Kalos

MIATA – Consensus Workshop in Washington



Conclusions 2/2

The assay harmonization efforts conducted over the past 5 years further led to the identification of several **critical experimental process steps**.

As a consequence of this **MIATA** was launched as a community driven reporting framework for T cell experiments.

Published reports of T cell experiments should include **sufficient information** on **all critical** test variables and process steps.

CIMT – Co-operations / Acknowledgements

Response Determination for ELISPOT Assays

Zoe Moodie (SHARP, Seattle) and Leah Price (University of New York)

ELISPOT – Serum Task Force

CRI/CIC (S. Janetzki)

Reporting Framework for T cell Assays - MIATA

Core Team Members, Panelists and all Contributors

Biomarker development in immunotherapy

iSBTC-FDA Biomarker Task Force

CIP – Team (WCF)

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Thank you for your attention

CIMT – 9th Annual Meeting 2011
May 25th-27th 2011 in Mainz

Targeting Cancer: Road-Map for Success

Confirmed speakers:
J. Mac Cheever (University of Washington)
Adrian Bot (MannKind Corporation)
Thorbald van Hall (Leiden Medical Center)
Lisa Butterfield (University of Pittsburgh)
Francesco Marincola (Trans-NIH Center for Human Immunology)
Jean-Yves Bonnefoy (Transgene)
Phil Greenberg (Seattle)
Vincent Brichard (GSK)
Axel Hoos (BMS)

Topics include:
Cellular Therapy
Therapeutic Vaccination
Therapeutic Antibodies
Immunomonitoring
New Targets
Cancer Biology
Immunotherapy
Immunosuppressive Mechanisms
Innate Immunity