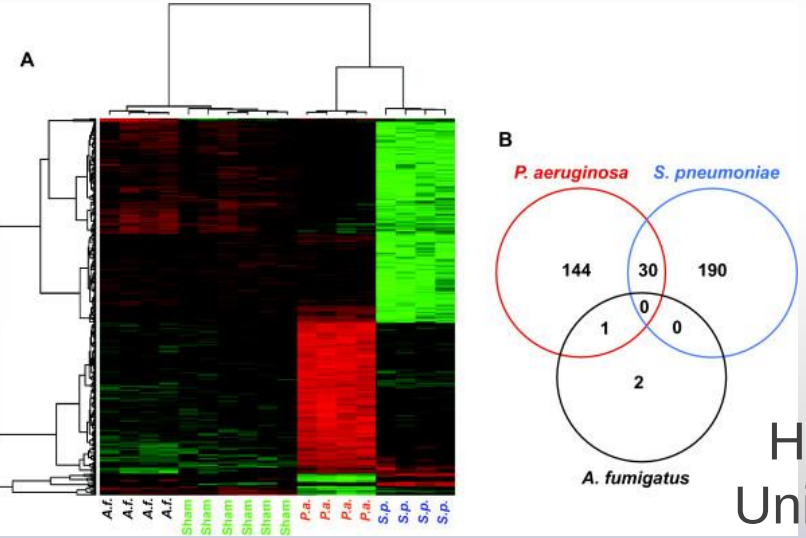
Analysis of Host Response signatures for diagnosis of invasive fungal infections
–
Immunreaktionen zur Diagnostik I



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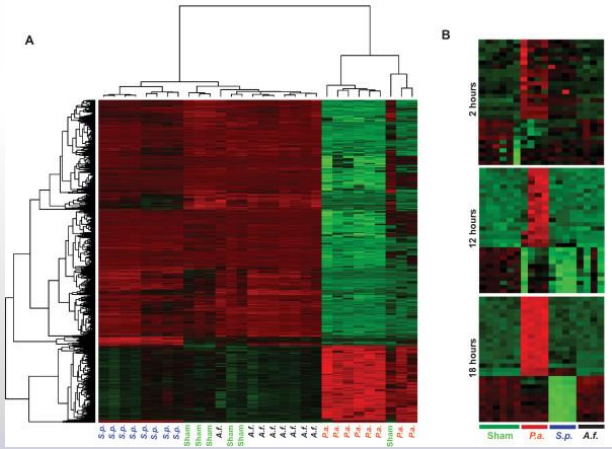
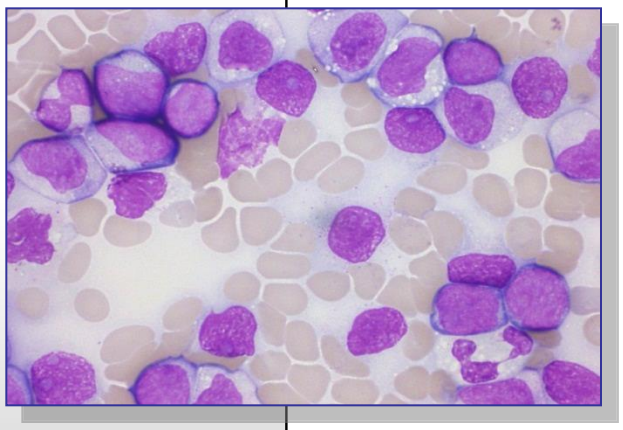


Aspergillus species – most frequent fungal pathogen in hematological high risk pts [1]



Distribution of organisms causing invasive fungal infection (n=995)

1. Kontoyiannis D et al., Clin Infect Dis 2010; 15, 50(8), 1091-1100

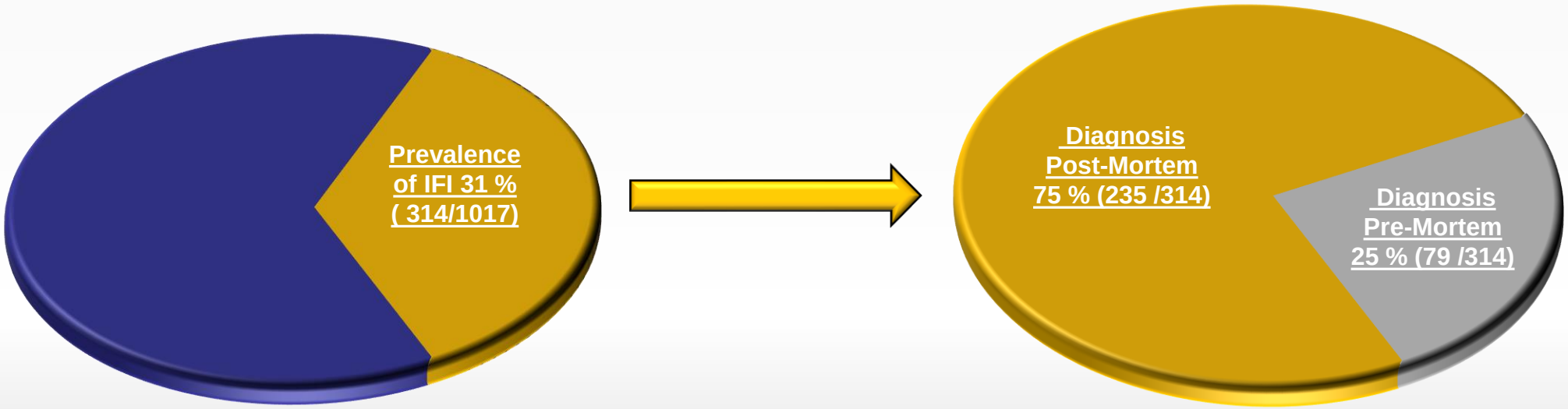
Diagnosing IA – The EORTC/MSG Criteria

Falldefinition	*Wirtsfaktoren*	*Clinical Features*	*Mycological criteria*
Proven **(=bewiesene Aspergillose)**	**+/-**	**+/-**	**Histopathologischer oder kultureller steril gewonnener Nachweis aus einer normalerweise sterilen Lokalisation**
Probable **(=wahrscheinliche Aspergillose)**	**+**	**+**	**Entweder** **a) Histopathologischer oder kultureller gewonnener Nachweis aus einer nicht-sterilen Lokalisation (z.B. BAL, Sputum, Nasenspülflüssigkeit)** **b) Positivität für GM** **c) Positivität für BDG**
Possible **(=mögliche Aspergillose)**	**+**	**+**	**-**
No IA **(=kein Hinweis auf IA)**	**+/-**	**+/-**	**-**

De Pauw et al., Clin Infect Dis 2008

Invasive aspergillosis in hematological pts …

- … is a life-threatening and *underdiagnosed* complication in patients at high risk receiving intensive chemotherapy and/or undergoing allo hematopoietic stem cell transplantation[2]



- Only 79/314 pts = 25 % with autopsy-proven IFI diagnosed premortem

2. Chamilos et al. Haematologica 2006; 91: 986-989

But we are getting better…….

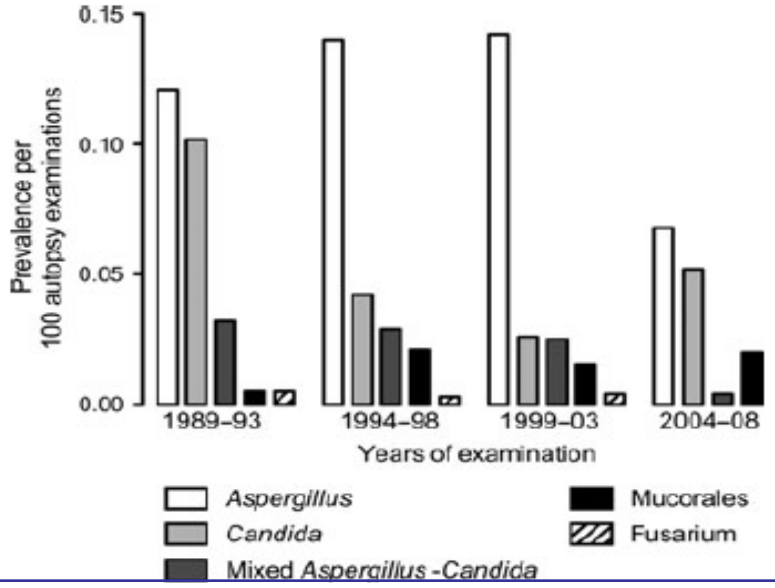
mycoses
Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Original article

Epidemiology and sites of involvement of invasive fungal infections in patients with haematological malignancies: a 20-year autopsy study

Russell E. Lewis,[1,*] Lizebeth Cahyame-Zuniga,[1] Konstantinos Leventakos,[1] Georgios Chamilos,[1] Ronen Ben-Ami,[1] Pheroze Tamboli,[1] Jeffrey Tarrand,[1] Gerald P. Bodey,[1] Mario Luna[2,†] and Dimitrios P. Kontoyiannis[1]

h, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA
es, Infection Control and Employee Health, The University of Texas M.D.

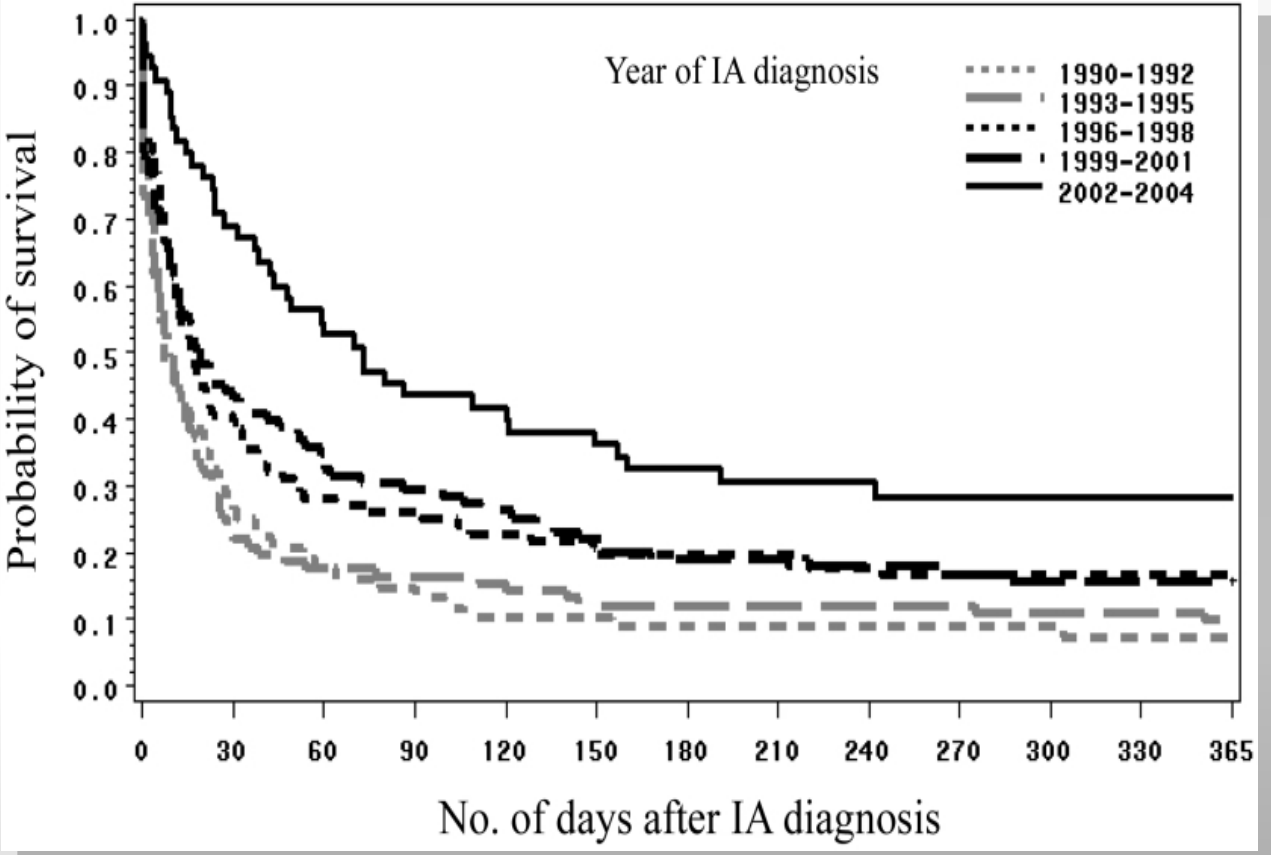


- 20-yr single center autopsy study
- declining autopsy rates, but fewer IFI
- after 2003, moulds majority of disseminated IFI at autopsy; IA decrease with mould active therapy
- increased prevalence of *Candida* in most recent time period
- improved antemortem diagnosis: 16 to 51%

Lewis R, Mycoses 2010

Invasive aspergillosis – mortality rates still high [3]

- Over the years decrease of mortality rates in IA pts
- Improvement possibly based on:
  a) increase of nonmyeloablative conditioning
  b) faster WBC recovery by use of peripheral blood stem cells
  c) improvement in diagnosing of IA
  d) new antifungal drugs



3. Upton et al. CID 2007; 44: 531-540

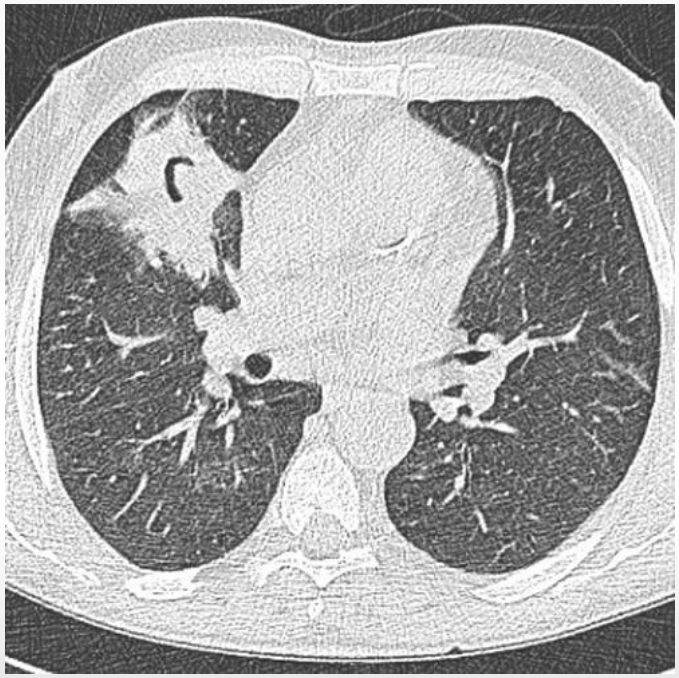
Diagnostics in IA – What to detect in IA ?

Host Evidence ??

Aspergillus Antibodies ??

Host Response

Host + Pathogen Evidence (Radiology)

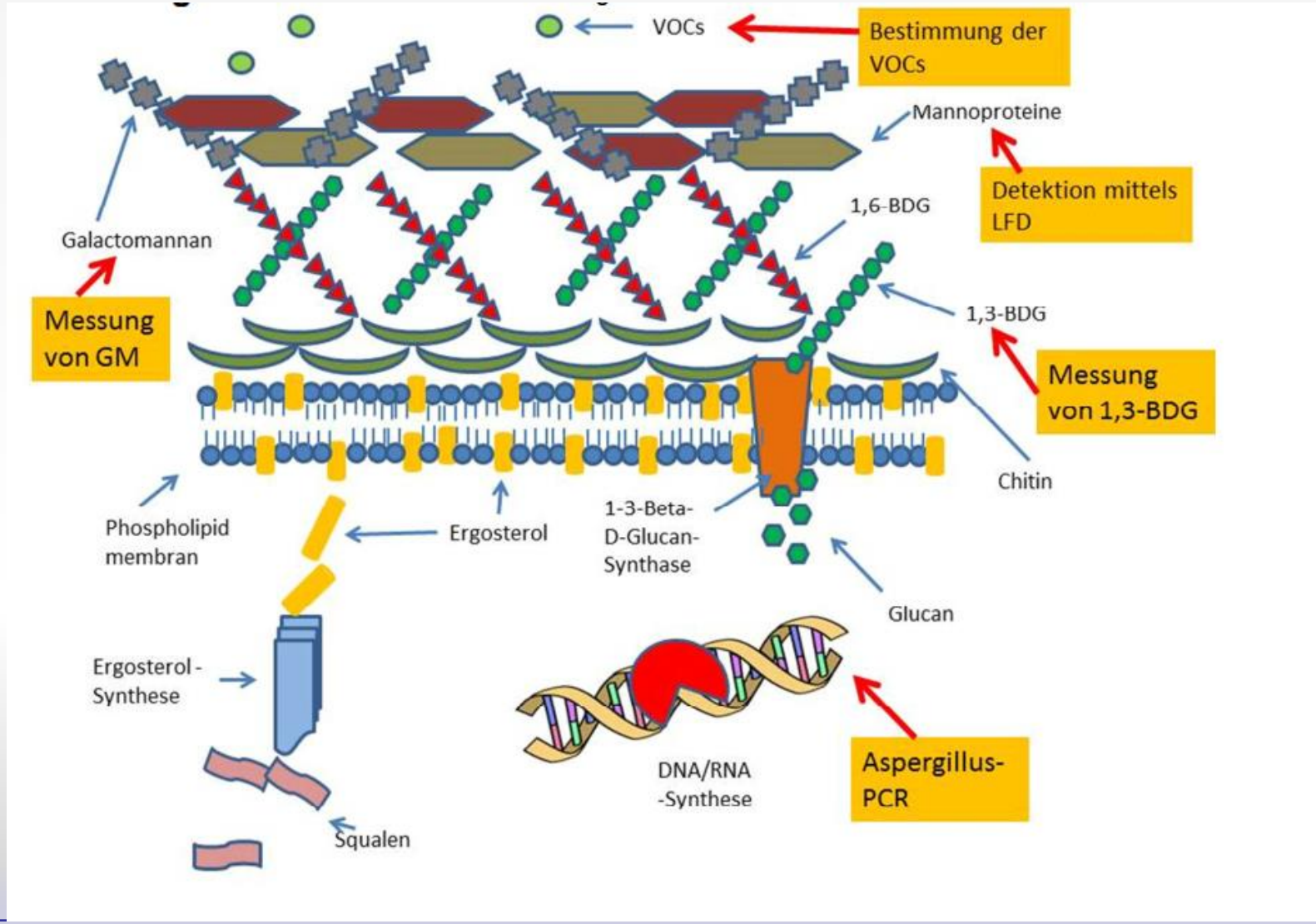


Pathogen evidence

- Histology
- Culture
- GM
- BDG
- Asp PCR

Host Response: The characteristic response pattern of an organism to an invading pathogen

Biomarkers in IA derived from the pathogens



Host Response Diagnostics

- comprehensive analysis of the host response to the infection caused by different microbial pathogens
- different classes of pathogens trigger specific pattern-recognition receptors (PRRs) differentially expressed on leukocytes
- a comprehensive molecular phenotype of these cells can be obtained using mould-specific T-cells, gene expression profiling (GEP) microarray analysis
- Advantages: a) Pathogens difficult to grow/detect might be detected (e.g. Mycobacteria, Moulds)

  b) Host response analysis might elucidate further potential diagnostic targets

Host Response Diagnostics – Already present in routine clinical practice ? Yes, they are……..

Table 3
Diagnostic performance of T-SPOT.*TB* and QFT-GIT assays for diagnosing active TB, including confirmed and probable TB

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
T-SPOT.*TB* assay	83 (75–89)	71 (60–81)	81 (72–87)	75 (64–84)
QFT-GIT assay	66 (56–74)	76 (66–85)	80 (69–87)	62 (51–71)

Values are shown as % (95% CI).

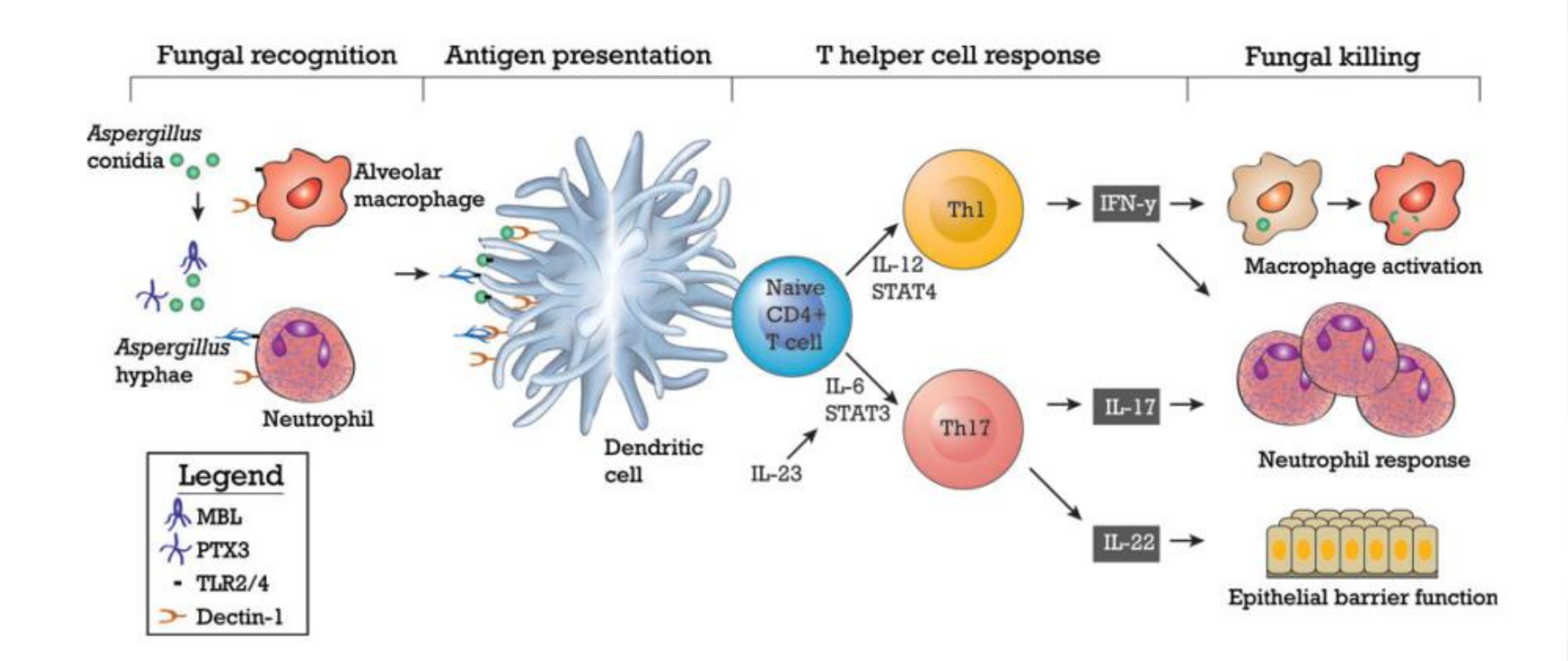
Clinical characteristics of patients with indeterminate results

Characteristics	QFT-GIT (n = 14)	T SPOT. *TB* (n = 4)
Mean age ± SD (years)	56.5 ± 15.7	57.5 ± 25.7
Male-to-female ratio	8:6	1:3
Underlying condition		
Malignancy	3 (21.4)	0 (0.0)
Solid cancer	2 (14.3)	0 (0)
Hematologic cancer	1 (7.1)	0 (0)
Diabetes mellitus	0 (0)	1 (25.0)
Steroid usage	5 (35.7)	0 (0)
Immunosuppressant usage	3 (21.4)	0 (0)
Lymphocyte <1500	9 (64.3)	1 (25.0)

- In the diagnosis of *M. tuberculosis infections* host-response-based analysis is the mainstay of non-invasive diagnostics with the TB-Elispot and the quantiferone assay

Lai CC, Diagn Microbiol Infect 2011

Detection of an IMD specific host response



Camargo, CID 2014

Genetic Factors in PRR affect IA incidence and survival – Polymorphisms in TLR4

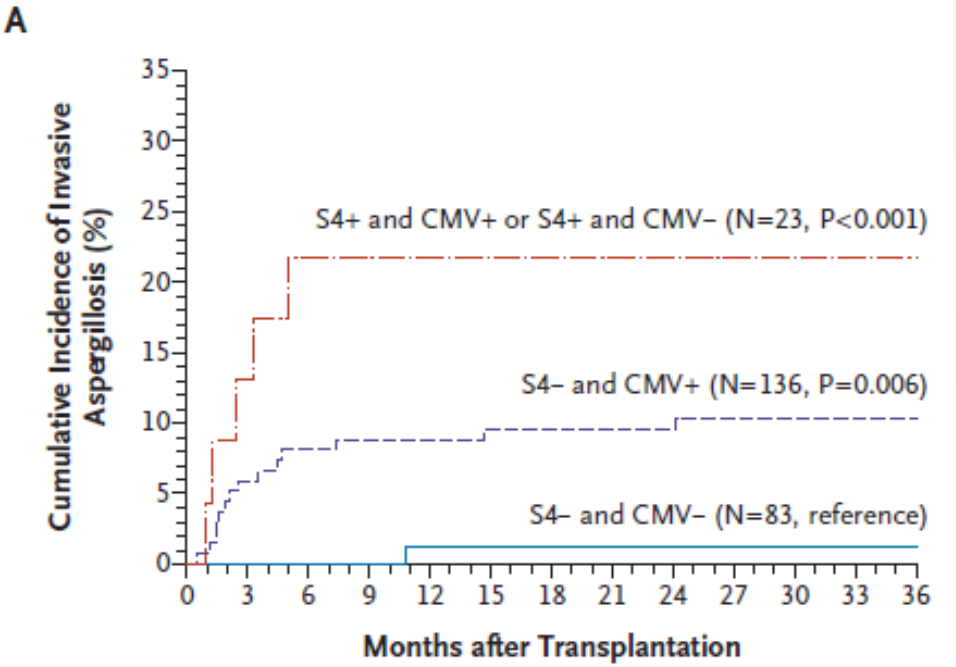


Table 4. Multivariate Analysis of Pretransplantation Risk Factors for Death Not Related to Relapse in the Discovery Study.

Variable	Adjusted Hazard Ratio (95% CI)	P Value
CMV seropositivity, S4 positivity, or both*	1.88 (1.10–3.20)	0.02
Underlying disease		
CML, chronic phase	Reference group	
Hematologic cancer		
First remission	1.60 (0.98–2.61)	0.06
Subsequent remission or relapse	0.55 (0.19–1.59)	0.27
Other underlying disease	1.11 (0.26–4.67)	0.89

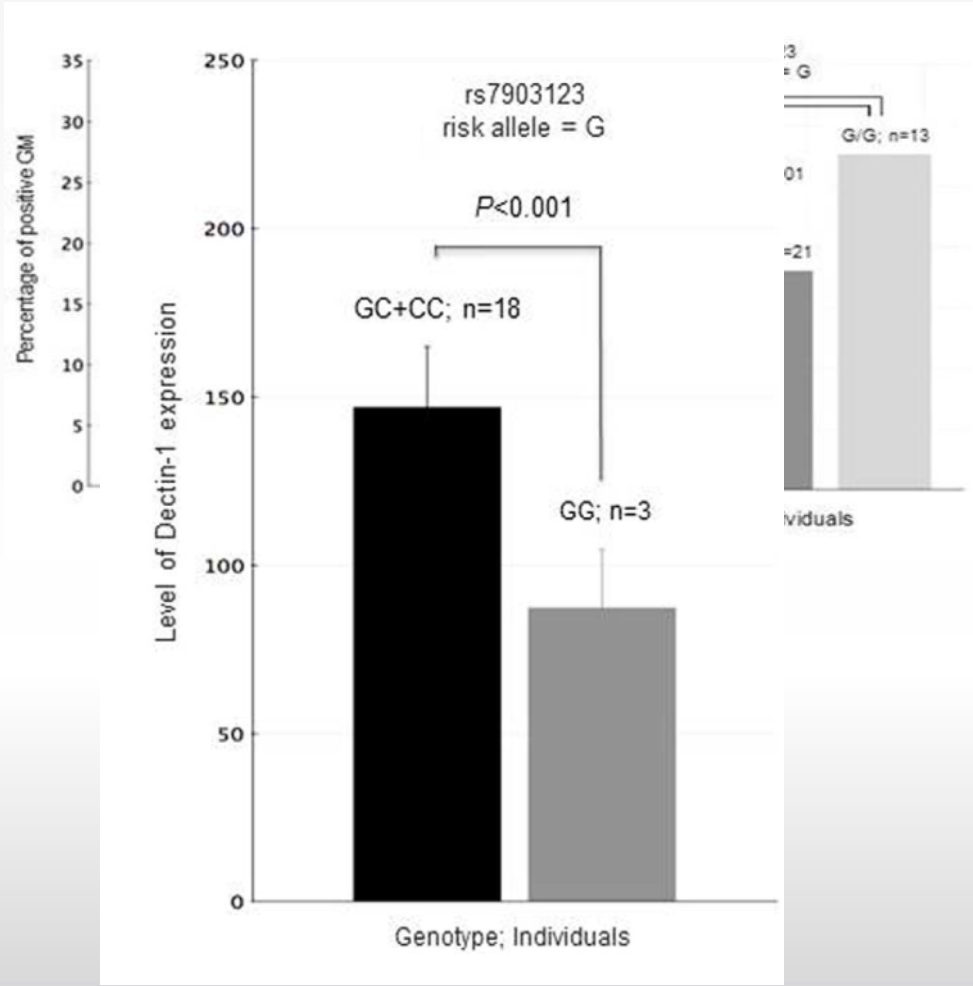
- Hematopoietic stem cell recipients transplanted with a specific TLR4 polymorphism (S4+) show increased rates of IA and have impaired outcome

Bochud P, NEJM 2008

Genetic Factors in Dectin-1 and DC-SIGN affect IA incidence

- Analysis of 182 pts with HM, 57 with proven/probable IA for SNPs in DC-SIGN, Dectin-1, Dectin-2, CCR2 and CCL2
- Significant association of SNPs with IA incidence was observed for DC-SIGN and Dectin-1
- Synergistic genetic effects were observed for SNPs in different PRRs

Dectin-1



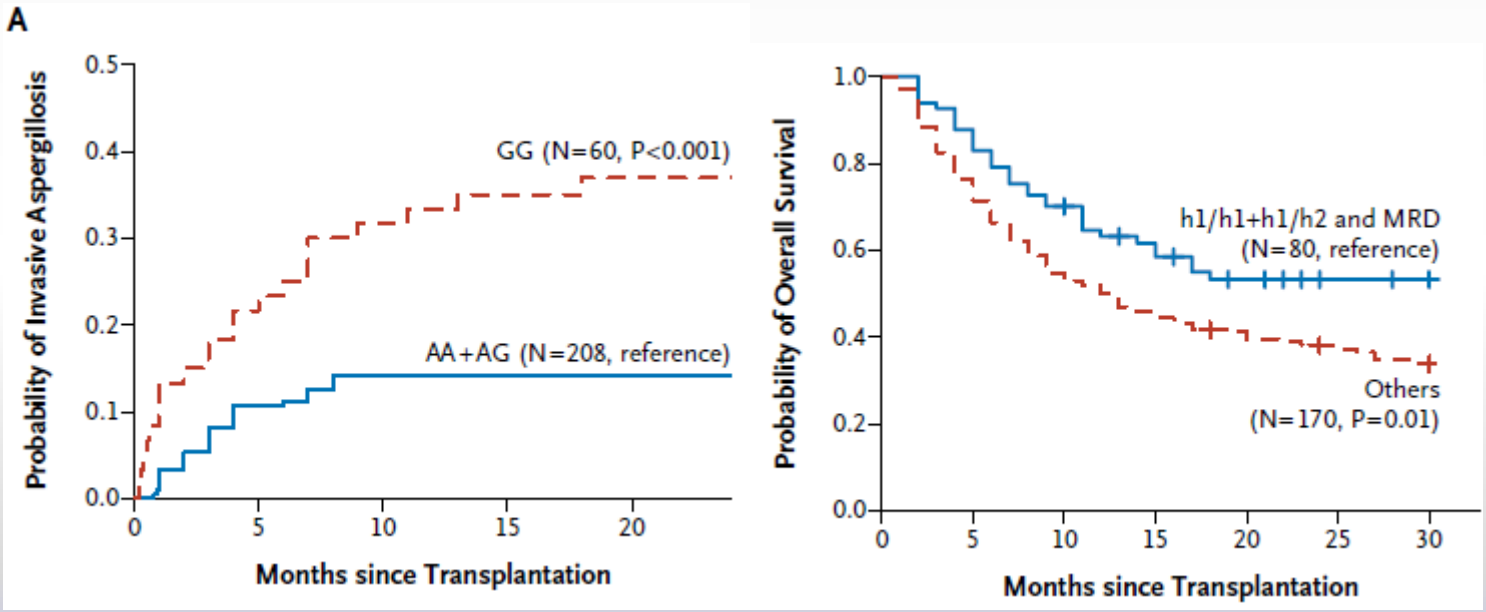
Sainz, PLOSone 2012

Genetic Factors in PRR affect IA incidence and survival – Polymorphisms in Pentraxin

Table 2. Multivariate Analysis of the Association of Donor *PTX3* Variants with the Risk of Invasive Aspergillosis among Transplant Recipients in the Discovery and Confirmation Studies.*

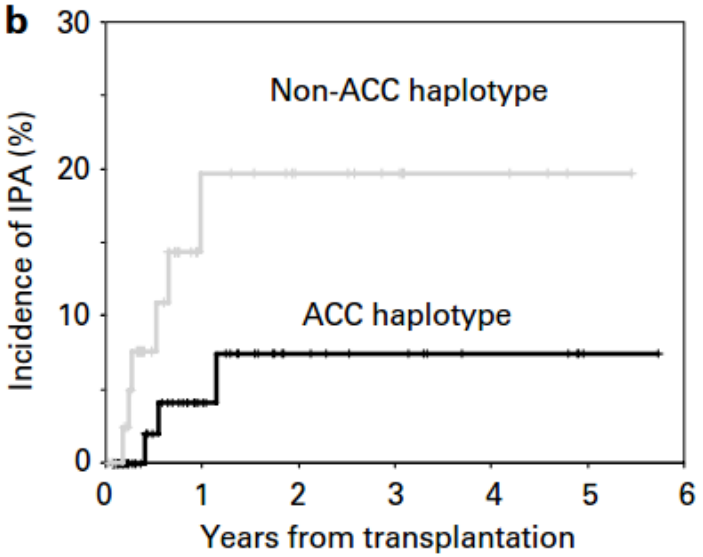
Donor *PTX3* Variant	Discovery Study (N=268)		Confirmation Study (N=330)	
	Adjusted Hazard Ratio (95% CI)†	P Value	Adjusted Odds Ratio (95% CI)‡	P Value
+281A/G SNP, GG genotype	2.92 (1.69–5.05)	<0.001	2.14 (1.20–3.80)	0.01
+734A/C SNP, AA genotype	2.62 (1.52–4.54)	<0.001	1.92 (0.91–3.04)	0.07
Haplotype h2/h2	3.08 (1.47–6.44)	0.003	2.78 (1.22–8.93)	0.03

- Polymorphisms in PTX3 affect IA incidence and outcome



Cunho C, NEJM 2014

Genetic Factors in PRR can also be protective – IL10-Polymorphisms



Haplotype	No of pts	IPA at 2 years (%)	*P*-value
ACC	3/61 (4.9%)	7.0±4.9	0.0307
Non-ACC	6/44 (13.6%)	19.7±7.7	

Figure 2 Incidence of IPA according to IL-10 promoter gene SNPs for all patients.

- In 105 BMT pts a protective effect was found for the IL-10 ACC haplotype regardless of chronic GvHD or HLA-disparity

Seo KW, BMT 2005

Analysis of Host factor PRRs for profiling risk of IA

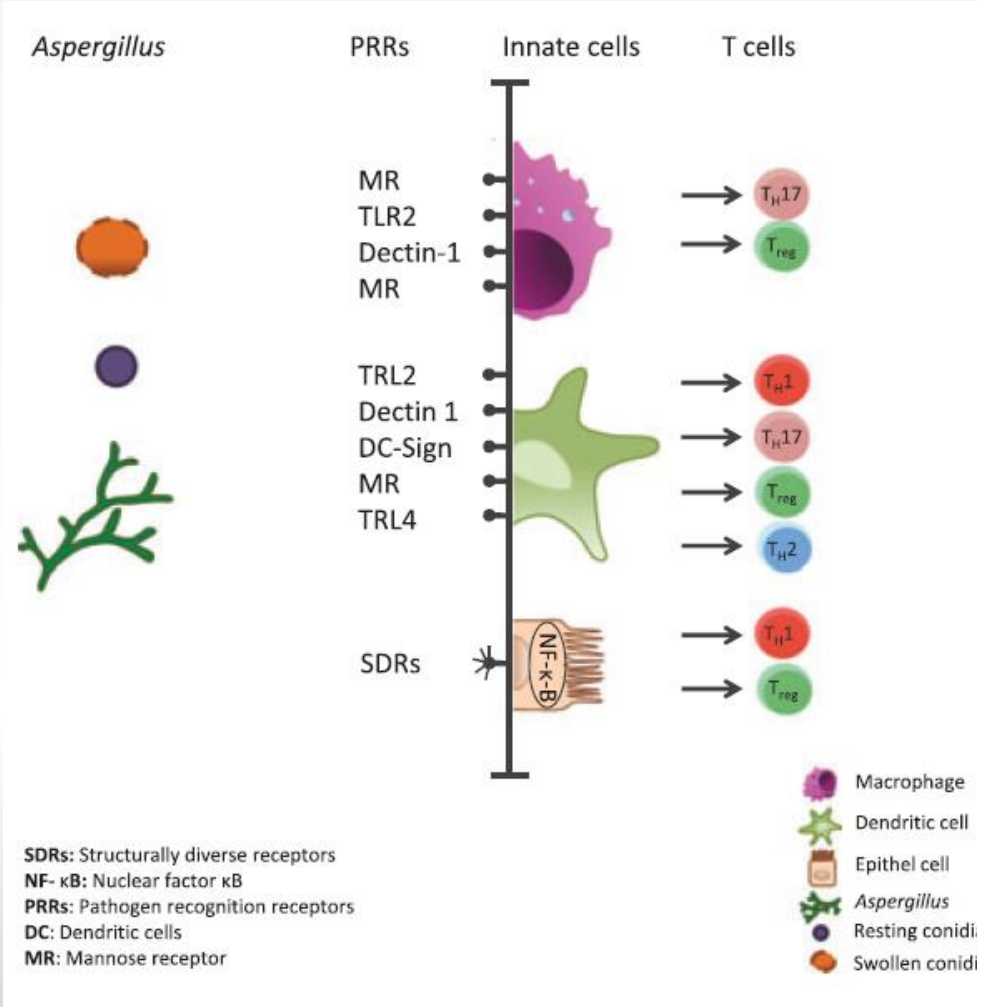
Table 3. Potential Biomarkers for the Risk of Invasive Aspergillosis in Immunocompromised Hosts

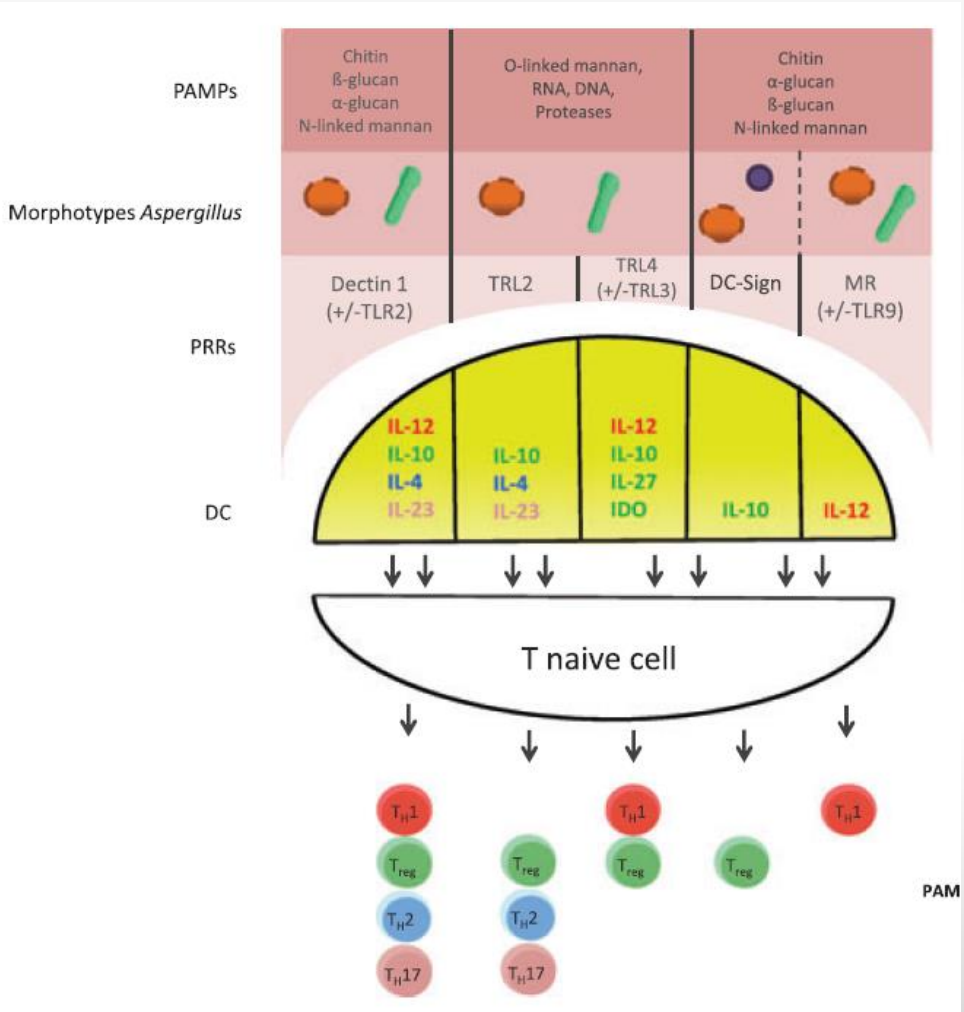
Biomarker	Main Role in IA	Knockout Mice	Human Data[a]	Technique/Specimen[b]
PTX3	Recognition of galactomannan	Highly susceptible	Genetic deficiency of PTX3 associated with risk of IA in HSCT; PTX3 levels are elevated in patients with IFI and normalize with successful antifungal therapy	ELISA/plasma or BAL
MBL	Recognition of monosaccharides	Not increased susceptibility	Reduced circulating levels of MBL seen in patients with IA	ELISA/plasma or BAL
DC-SIGN	Recognition of galactomannan	ND	Polymorphisms in *DC-SIGN* increase susceptibility to IA	Flow cytometry/whole blood or PBMCs
Dectin-1	Recognition of β-glucan; promotes Th17 response	Highly susceptible	Polymorphisms resulting in reduced dectin-1 expression increase susceptibility to IA	Flow cytometry/whole blood or PBMCs
TLR4	Fungal recognition and phagocyte activation	Increased fungal burden	Donor polymorphisms in *TLR4* increase the risk of IA after HSCT	Flow cytometry/whole blood or PBMCs
TLR2	Fungal recognition and phagocyte activation	Increased fungal burden	Polymorphisms in *TLR1* and *TLR6*, the TLR2 coreceptors, are associated with IA	Flow cytometry/whole blood or PBMCs
TLR3	Promotes protective cytotoxic T-cell responses	Highly susceptible	Donor polymorphisms in *TLR3* increase the risk of IA after HSCT	Flow cytometry/whole blood or PBMCs
Th17	Promote neutrophil recruitment via IL-17 and epithelial barrier functions via IL-22	ND	Polymorphisms in components of the Th17 pathway (eg, IL-1β, IL-23R, and STAT3) influence susceptibility to IA	Flow cytometry/whole blood or PBMC Immunoassay/ supernatant

DC-SIGN	Hematological malignancies	c.2797	A/G	2.75 (1.27–5.95)	Study included chemotherapy-treated patients and HSCT recipients	[29]

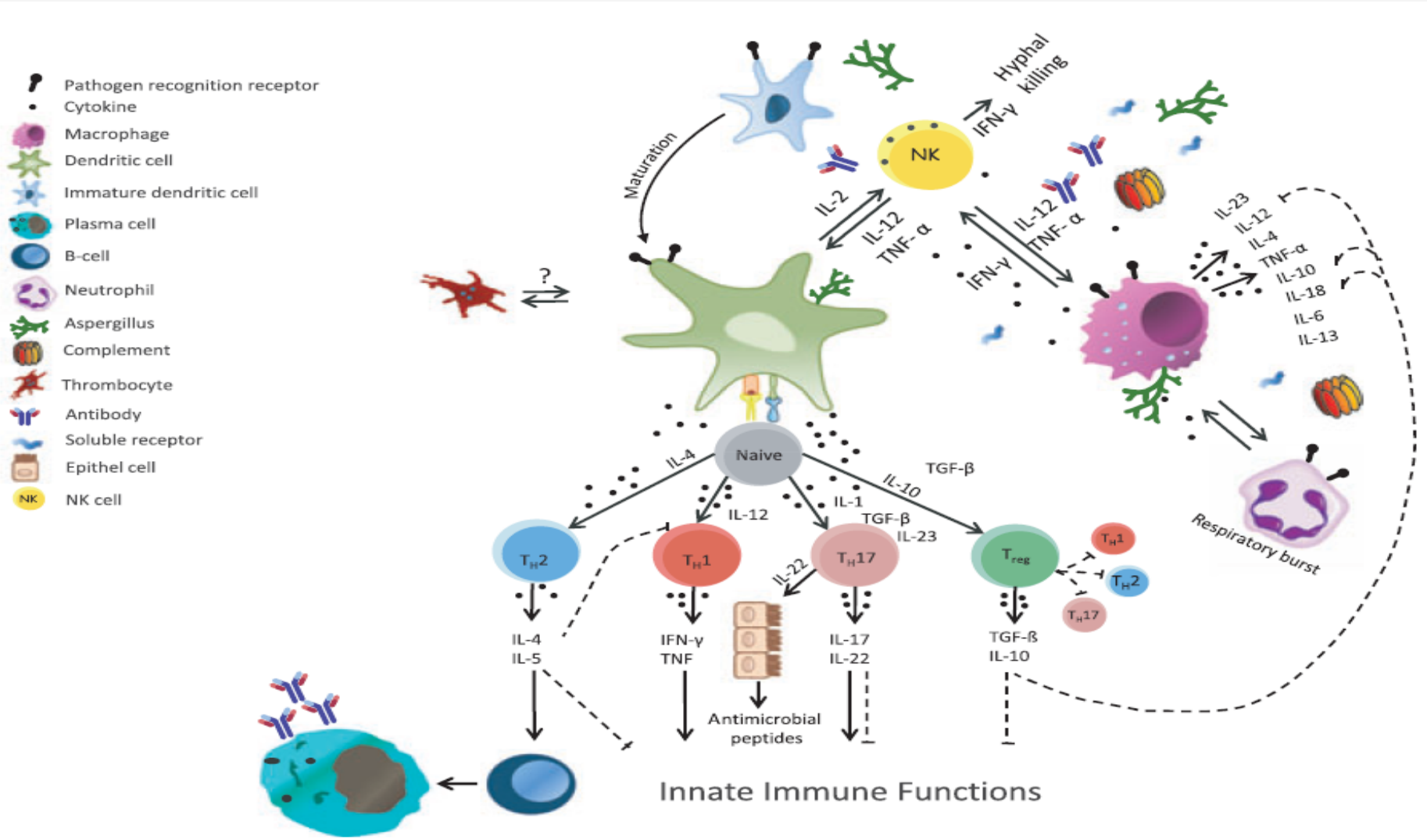
Camargo J, CID 2014

Host response – Background in IA



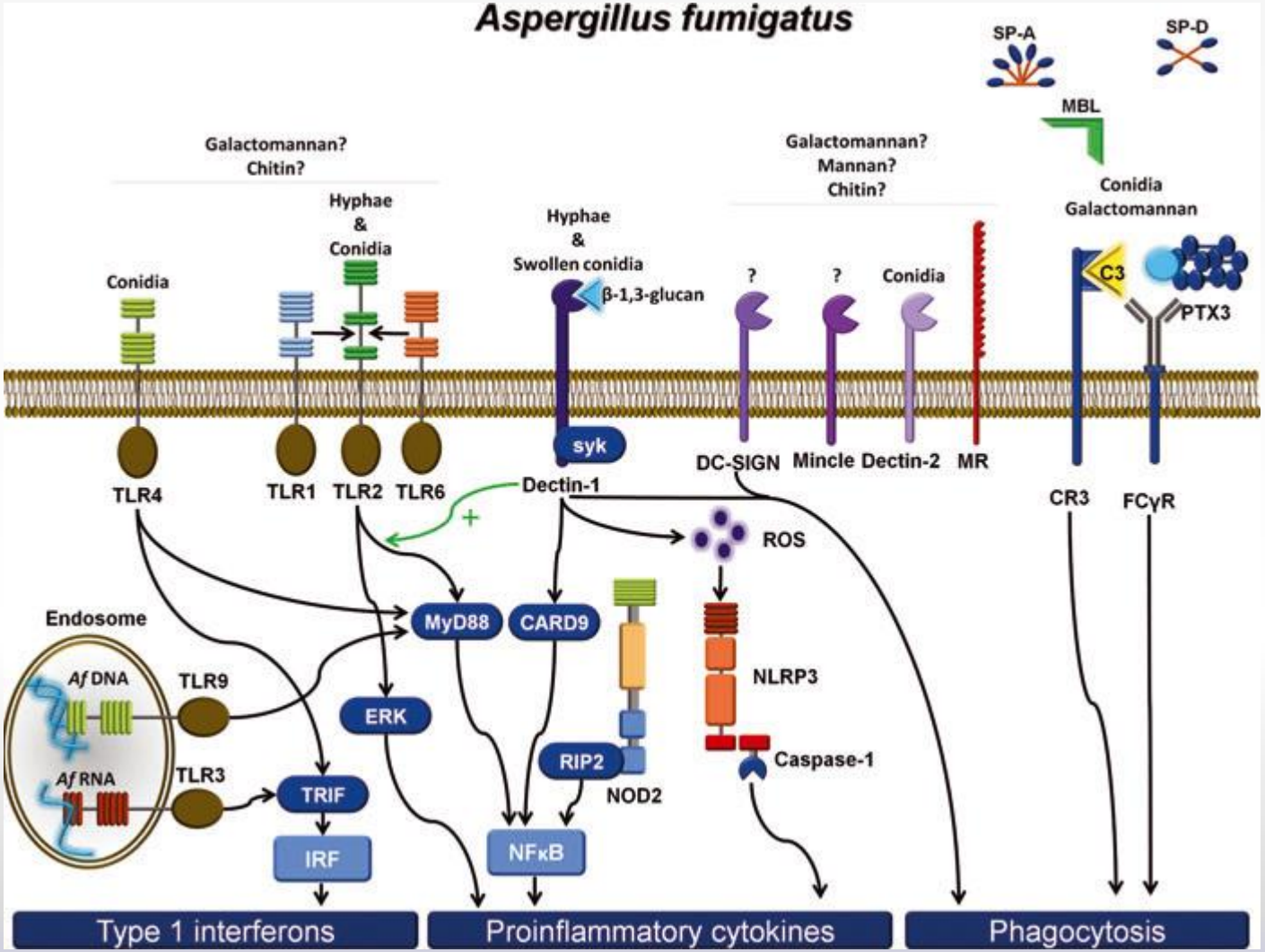


Host response – Background in IA II



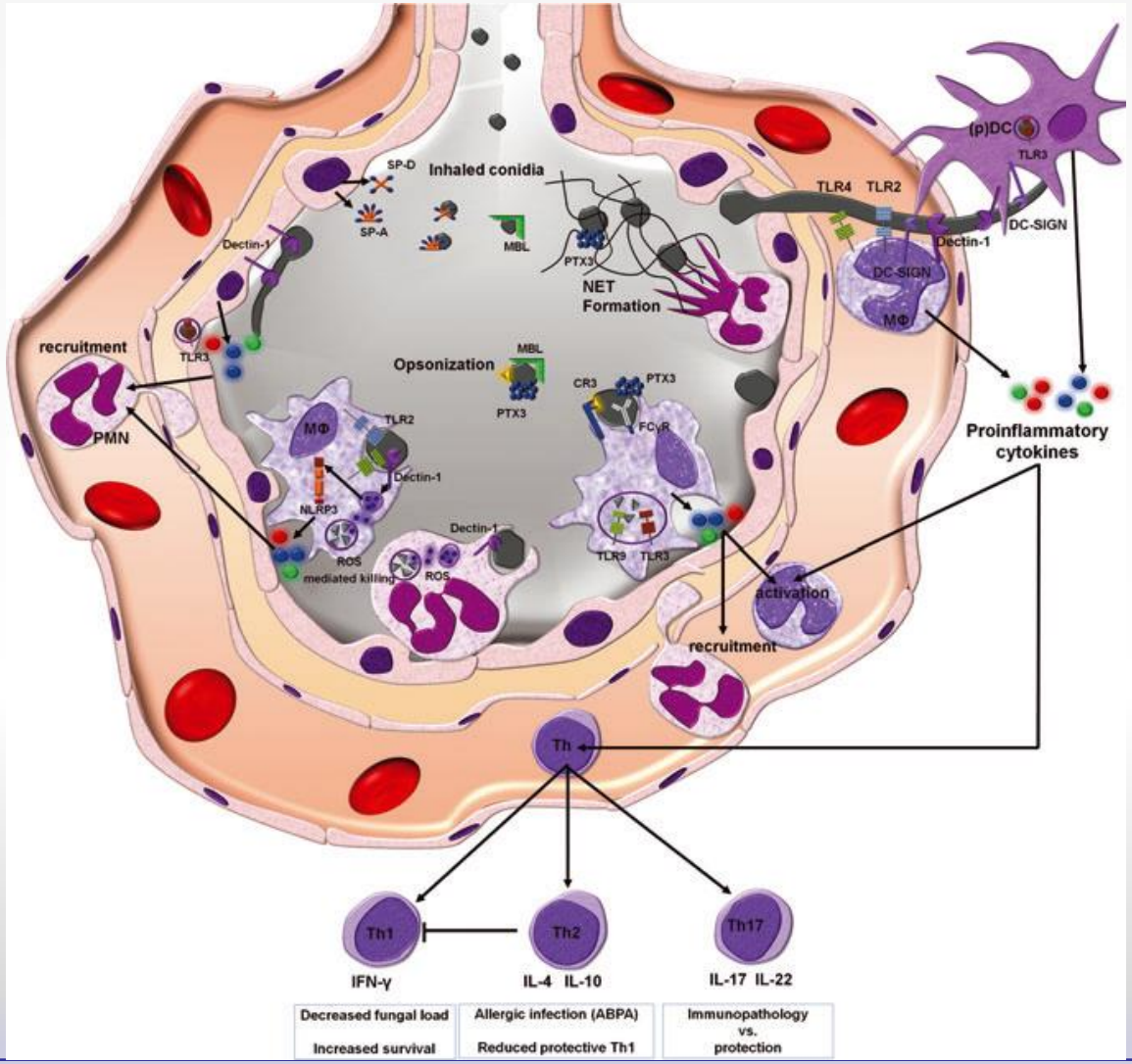
Lass-Floerl C, Mycoses 2013

Host response – Background in IA III



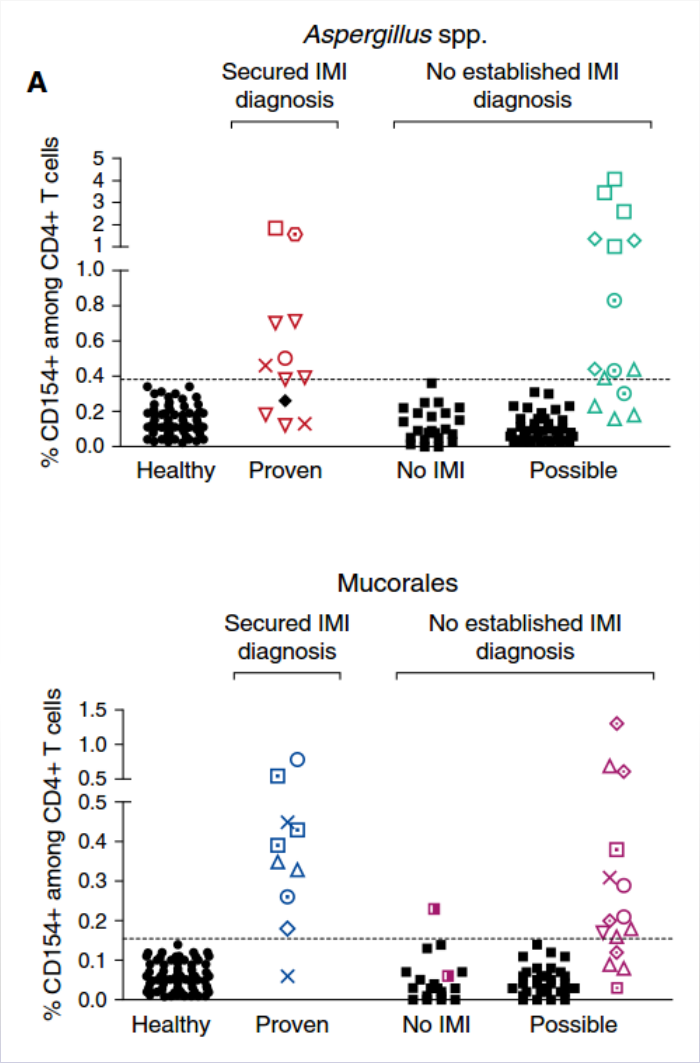
Gressnigt MS , Ann N.Y. Acad Sci 2011

Host response – Background in IA III

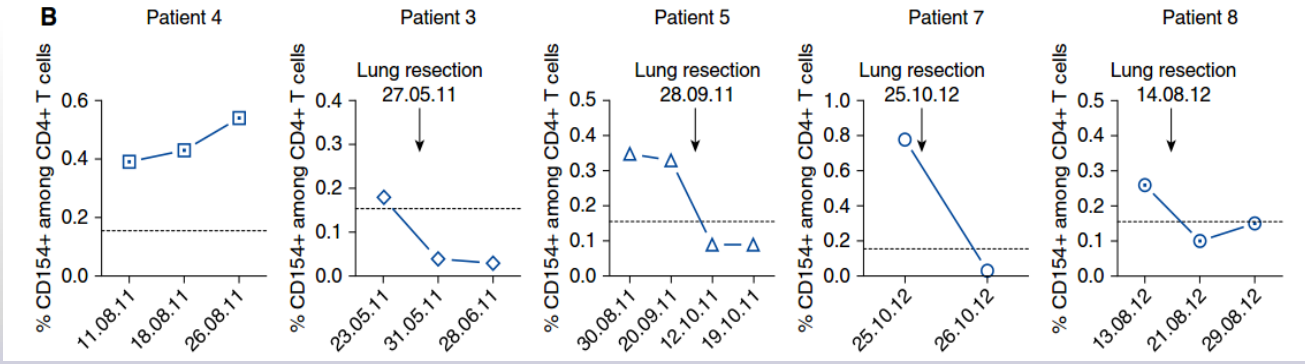


Gressnigt MS , Ann N.Y. Acad Sci 2011

Host response – Using mould-specific T-cells for diagnosis

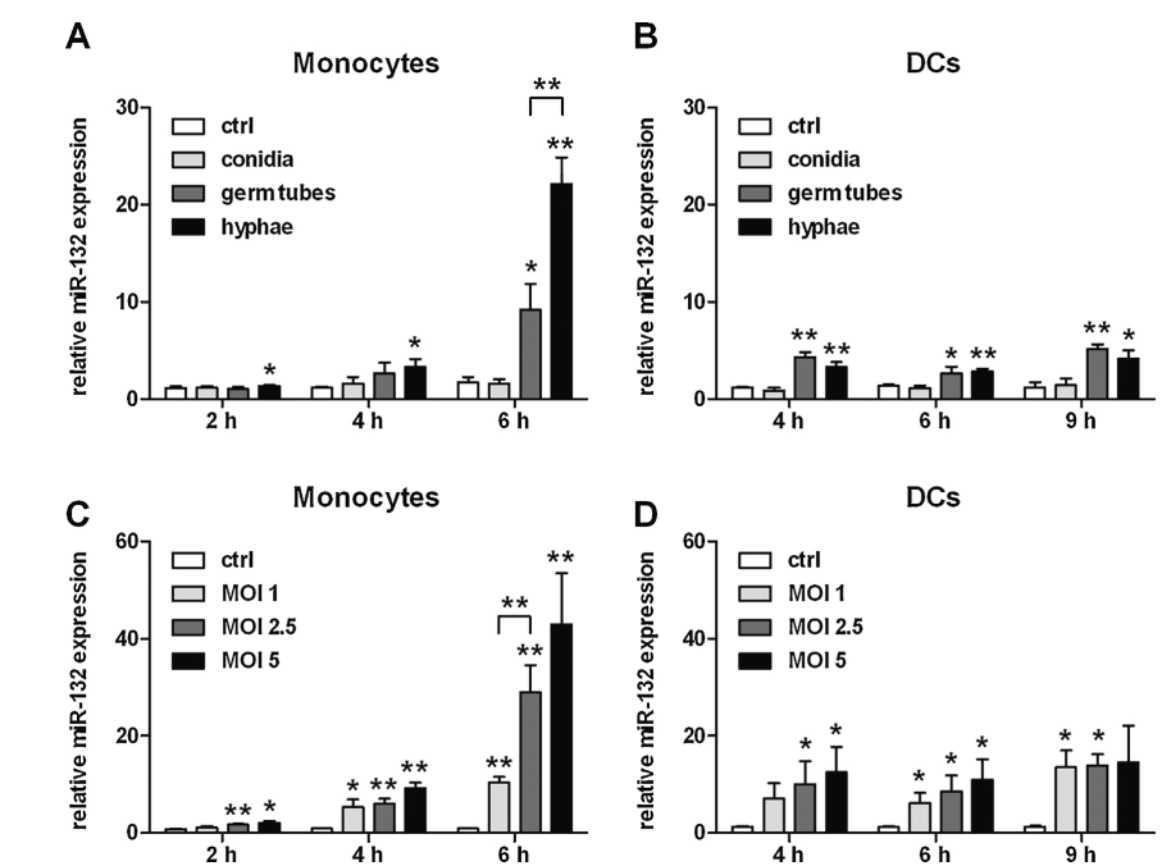


- Using an established assay 69 pts were assesed for mould (Aspergillus/mucorales) – reactive T-cells based on upregulation of CD154
- ROC analysis revealed a cutoff of 0.39 % CD154+ among CD4 cells as being suggestive of IA for *Mucorales* a cutoff of 0.16 % was found
- Sensitivity 90%, specificity 80 %



Host response –inoculation with *Aspergillus* leads to increase in miRNA 132 expression

- In a preclinical model confrontation of monocytes and DC cells leads to increase in microRNA 132 expression
- Inoculation with LPS did not lead to increase suggesting a fungal-specific reaction



In bacterial brain abscesses differential expression of inflammatory cytokines is observed for different species

- In 90 pts with bacterial brain abscesses expression of inflammatory cytokines was measured by ELISA and qPCR
- Differential expression was observed for different bacterial species
- Local expression was higher than systemic concentrations

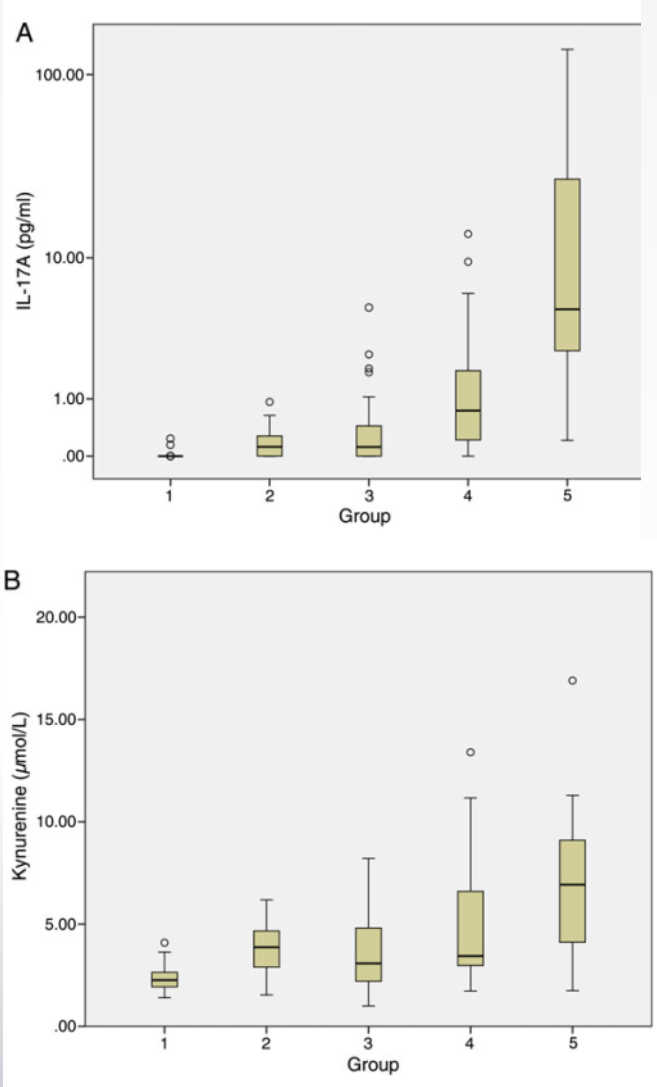
Cytokines	PCR				p value	Between group significance
	SA (n = 13)	SI (n = 08)	BF (n = 08)	EC (n = 07)		
TNF-α	5.5 ± 0.63	5.4 ± 0.88	2.7 ± 0.48	2.7 ± 0.35	<0.05	a,b,c,d
IFN-γ	1.9 ± 0.15	1.6 ± 0.16	1.01 ± 0.23	0.64 ± 0.09	<0.001	a,b,d
IL-1β	7.9 ± 0.25	6.3 ± 0.57	2.0 ± 0.33	2.4 ± 0.23	<0.001	a,b,c,d
IL-4	–	–	–	–	–	–
IL-10	2.9 ± 0.15	2.3 ± 0.26	6.2 ± 0.39	7.2 ± 0.37	<0.001	a,b,c,d
IL-17	3.9 ± 0.45	3.1 ± 0.31	1.1 ± 0.18	1.0 ± 0.16	<0.001	a,b,c,d
IL-23	1.1 ± 0.10	1.3 ± 0.15	0.81 ± 0.08	0.70 ± 0.06	<0.05	b,c,d

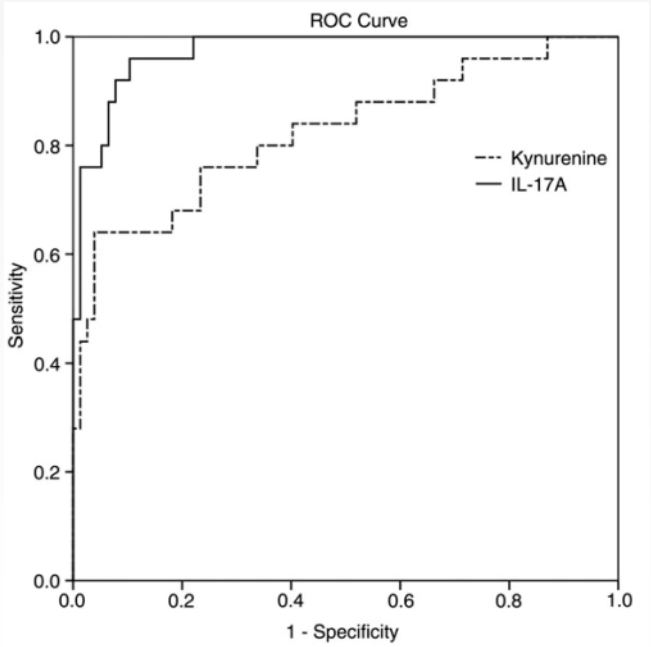
Concentration of cytokines (pg/ml) in abscess (local) with blood (systemic) (n = 48) [mean ± SD].

Cytokines	Local cytokine response (abscess)	Systemic cytokine response (blood)	Local vs systemic cytokine response p value
TNF-α	76.6 ± 4.7	28.6 ± 2.4	<0.001
IFN-γ	36.9 ± 2.8	21.5 ± 1.1	<0.001
IL-1β	14,248.7 ± 909.7	28.3 ± 1.5	<0.001
IL-4	–	–	–
IL-10	103.0 ± 5.2	53.1 ± 5.0	<0.001
IL-17	94.3 ± 4.4	0.00	–
IL-23	37.1 ± 2.1	17.1 ± 0.73	<0.001

Bajpai A, J Neuroimmun 2014

Significant increase in IL17a and Kynurenine in Candidemia compared to non-candidemia infections



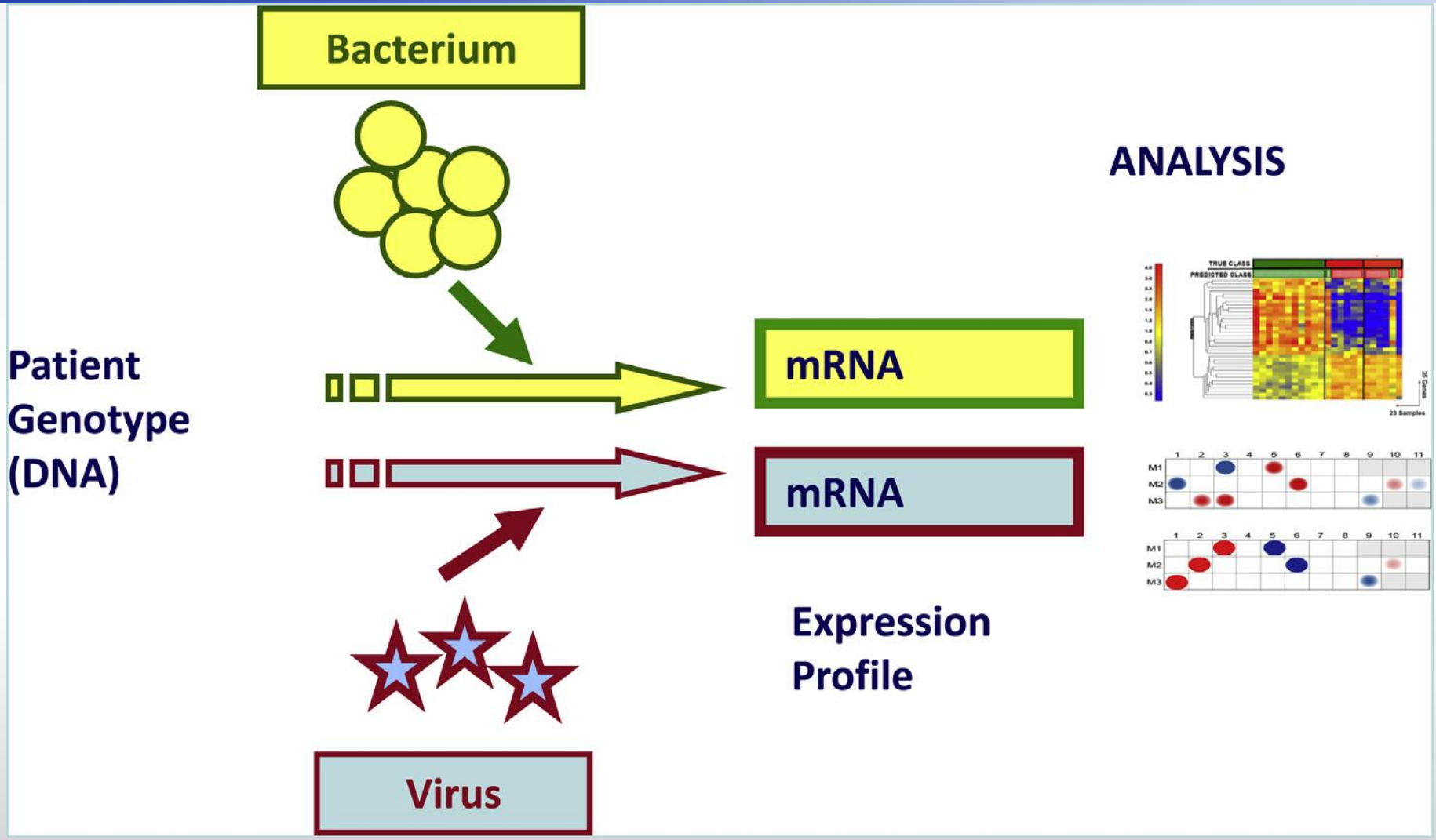


- Dectin-1, TLR2, TLR4, Tregs, IL-6, IL-10, IL17A, IL-22, IL-23, IFN-ɣ, Kynurenine and tryptophane were quantified in ICU pts (n=131)

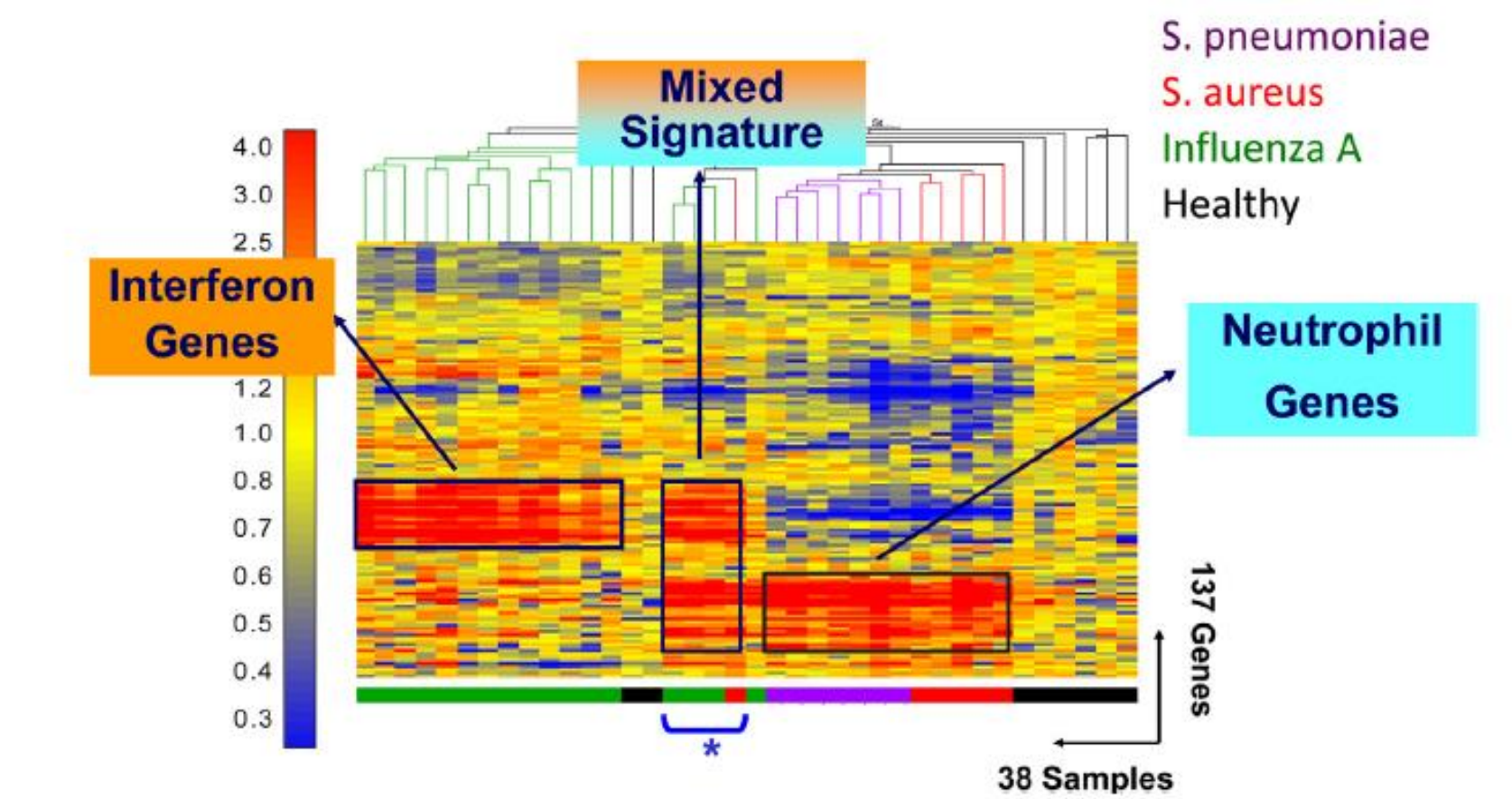
- comparison of candidemia and non-candidemia
- in ctrls 6/44 had other positive blood cultures

Krause R, J Infection 2015

Transcriptional Profiling of Host response



Transcriptional Profiling of Host response using GEP



- GEP is able to discriminate between different pathogen species based on specific expression signature of blood leukocytes

Ramilo O, Blood 2007

Transcriptional Profiling via GEP is superior to PCT for differentiating viral from bacterial LRTI

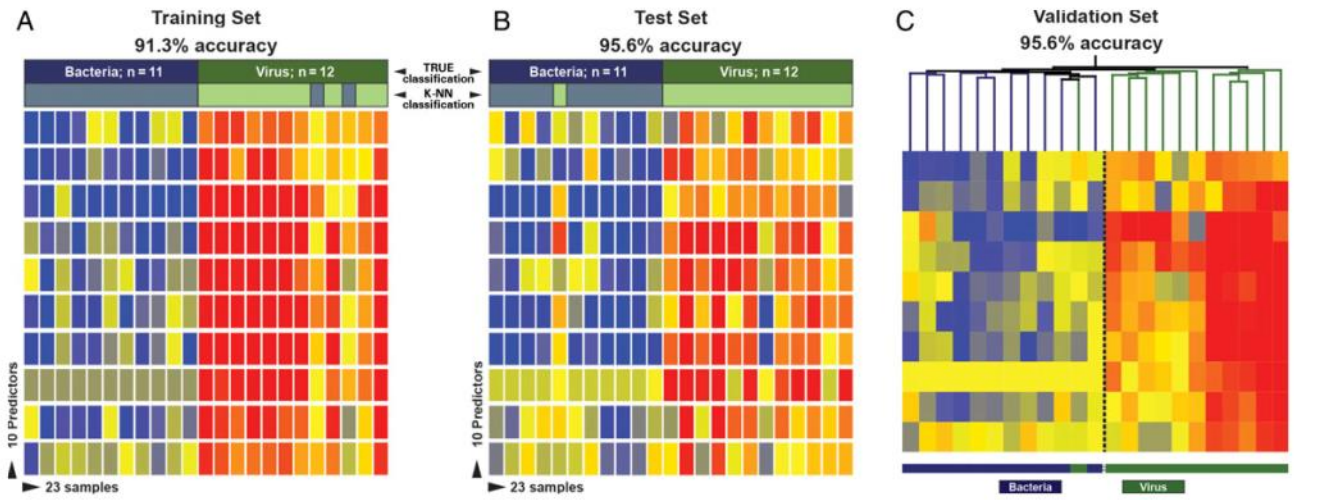


Table 2. Classifier Genes That Best Discriminate Bacterial From Viral LRTI[a]

Gene	Bacterial LRTI	Viral LRTI
BTN3A3	0.40	1.21
IFI27	2.16	57.49
RSAD2	0.73	14.24
KIAA1618	0.86	2.73
OAS2	0.85	3.51
IFIT3	0.64	4.35
IFI44	0.83	9.17
OASL	1.11	5.22
IFIT2	0.86	2.56
PARP9	1.35	2.62

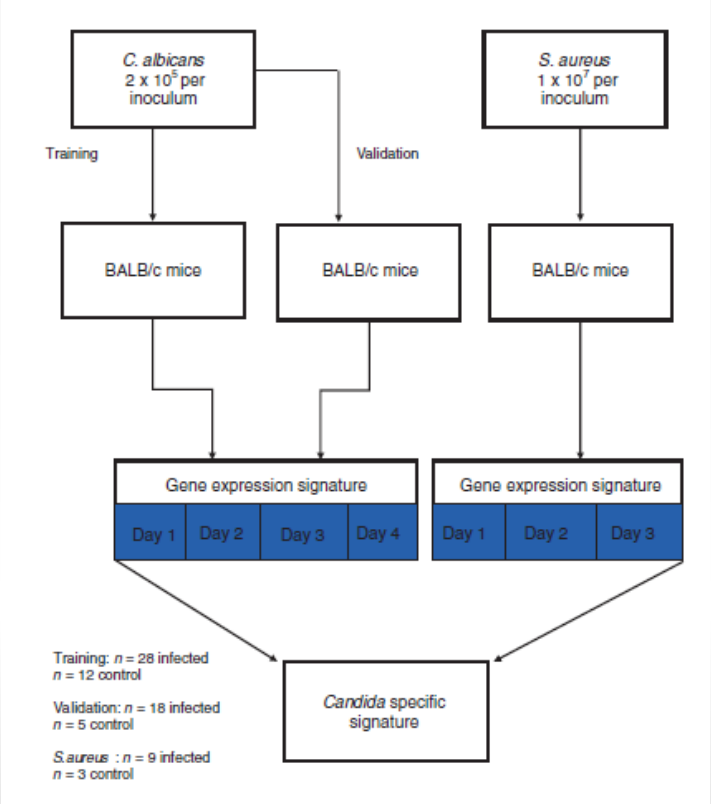
Table 3. Comparative Sensitivity and Specificity of PCT and Classifier Genes to Discriminate Between Viral and Bacterial LRT

Method	Correct, No. (%)	Incorrect, No. (%)	Sensitivity (95% CI), %	Specificity (95% CI), %
PCT (n = 55)				
Bacterial	8 (38.1)	13 (61.9)	38 (18–62)	91 (76–98)
Viral	31 (91.2)	3 (8.8)		
Total	39 (70.9)	16 (29.1%)		
Classifier genes (n = 58)				
Bacterial	21 (95.5)	1 (4.5)	95 (77–100)	92 (77–98)
Viral	33 (91.7)	3 (8.3)		
Total	54 (93.1)	4 (6.9)		

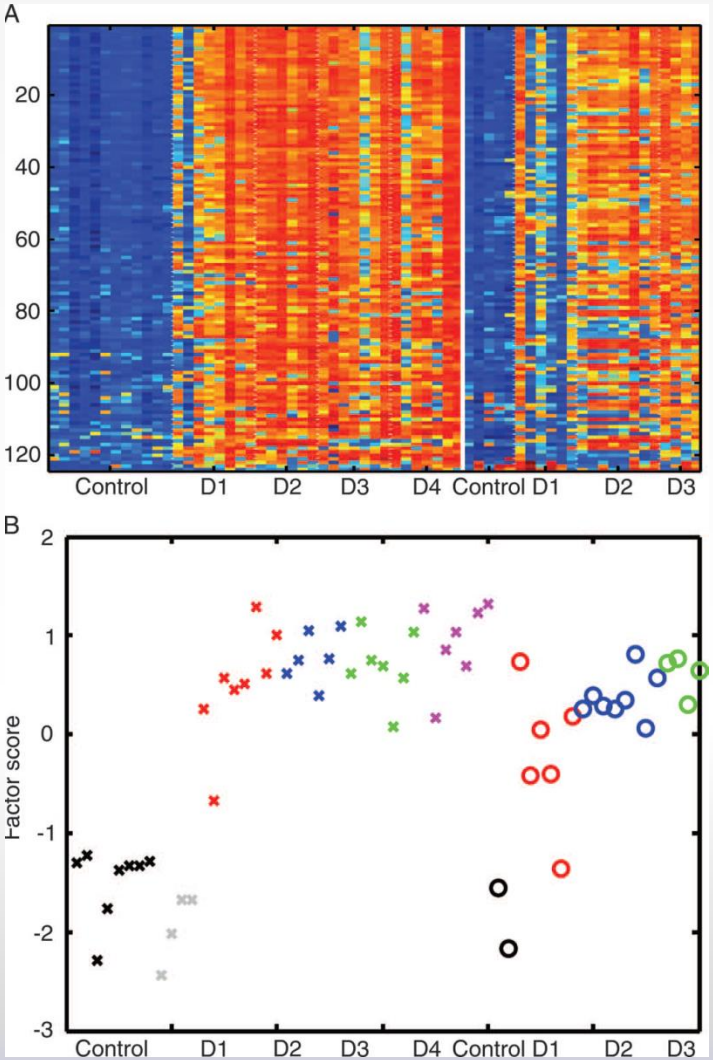
- Prospective study in adult pts hospitalized for LRTI (118 pts, 40 ctrls)
- Immunocompromised pts and pts under antibiotics prior to admission were excluded
- GEP was superior in discriminating between bacterial and viral lower respiratory tract infections compared to procalcitonin

Suarez NM, J Infect 2015

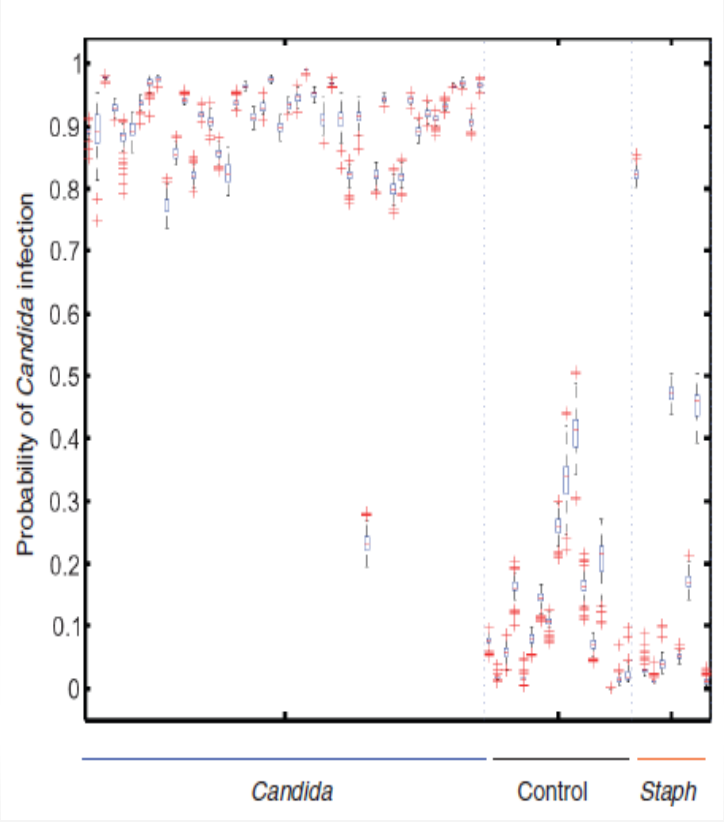
Transcriptional Profiling of Host response in invasive fungal infections - Candidemia

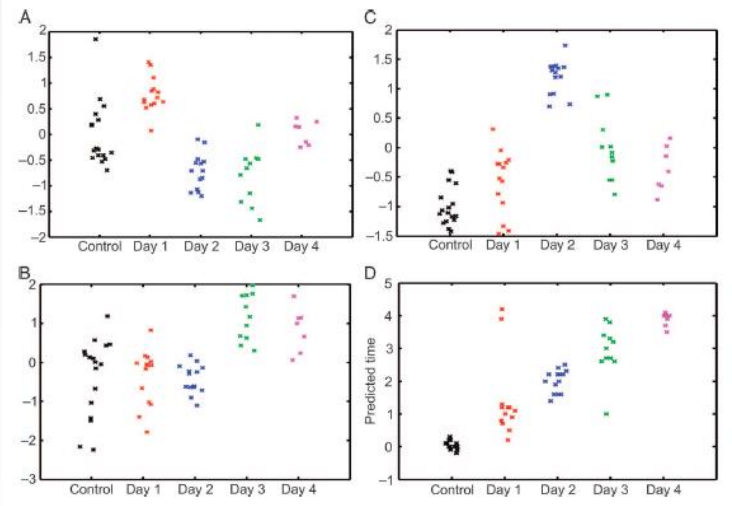


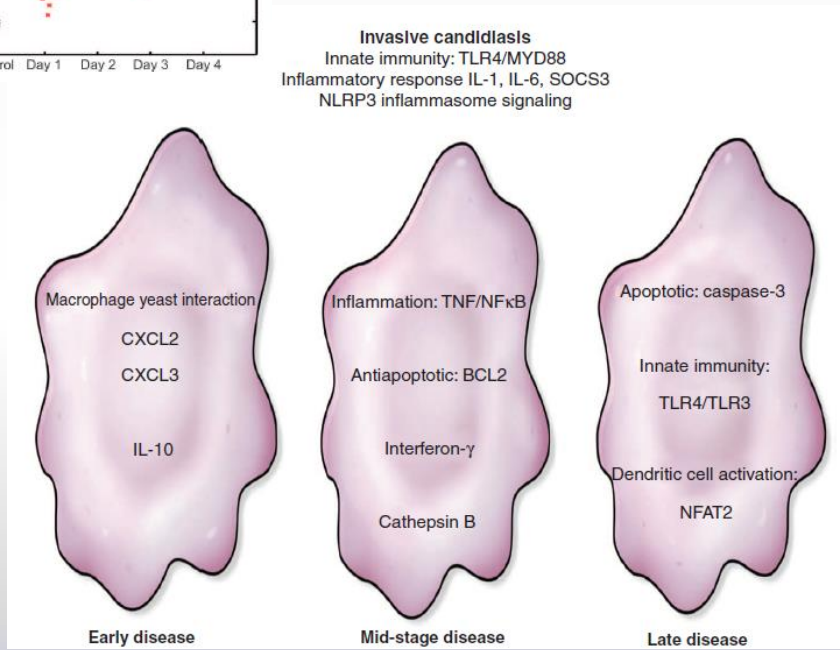
- Preclinical data show that candidemia has a specific gene expression signature compared to healthy controls and *S. Aureus* bacteremia



Transcriptional Profiling of Host response in invasive fungal infections - Candidemia







- Candidemia shows characteristic GEP patterns that change dynamically over time in relation to treatment or progressive disease

Zaas AK, Sci Trans Med 2010

Transcriptional Profiling reveals differentially expressed genes involved in immunoprocesses

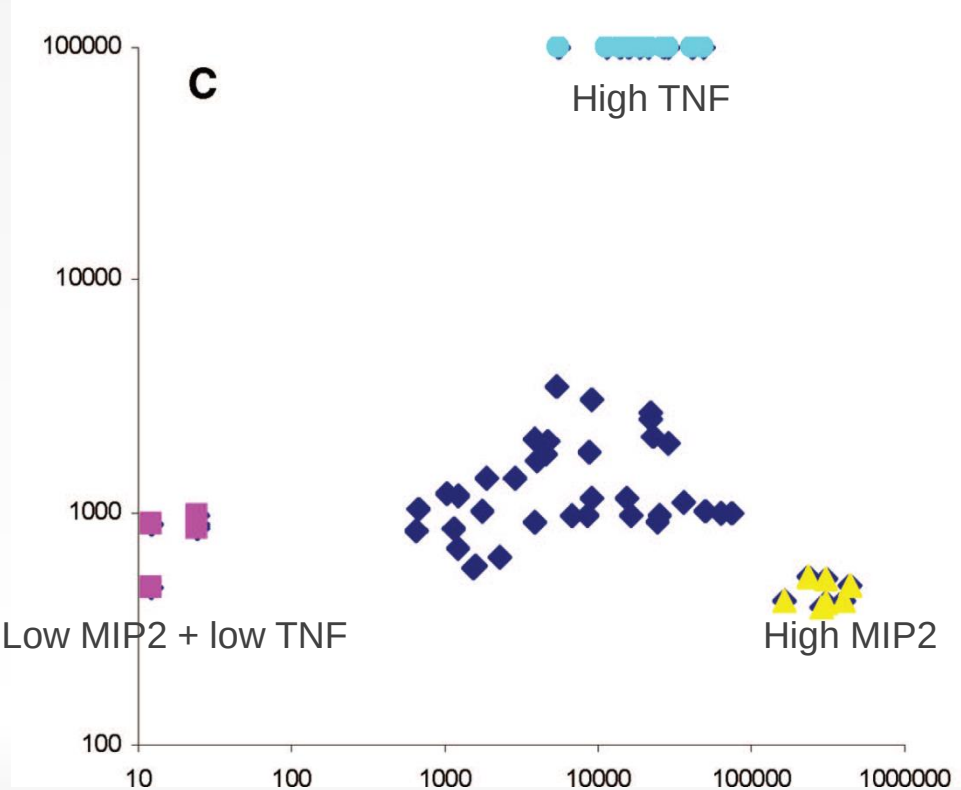
CD33 antigen
CD52 molecule
chemokine-like factor
cytokine receptor-like factor 2
colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)
colony stimulating factor 3 receptor (granulocyte)
Epstein-Barr virus induced gene 3
Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide
Fc fragment of IgG, low affinity IIa, receptor (CD32)
GLI pathogenesis-related 1 (glioma)
G protein-coupled receptor 97
inducible T-cell co-stimulator ligand
interferon induced transmembrane protein 1 (9-27)
interferon induced transmembrane protein 2 (1-8D)
interferon induced transmembrane protein 6
inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein
interleukin 10 receptor, beta
interleukin 13 receptor, alpha 1
interleukin 1, beta
interleukin 1 family, member 9
interleukin 1 receptor, type II
interleukin 1 receptor accessory protein
interleukin 1 receptor antagonist
interleukin 8 receptor, beta
immunoresponsive 1 homolog (mouse)
kelch-like 2, Mayven (Drosophila)

- Candidemia shows gene expression patterns that upregulate specific signatures involved in immunologic processes

- Construction of a GEP model that predicted 98 % of Candida infections in a validation cohort, Performance características showed a sentitivity of 96 % and specificity of 100 %

Zaas AK, Sci Trans Med 2010

Transcriptional Profiling of Host response in sepsis



- GEP is able to define subgroups with higher mortality rates and prone to suffer from severe sepsis

Table 3 Differences in expression of relevant pharmacogenes between the two sepsis subtypes

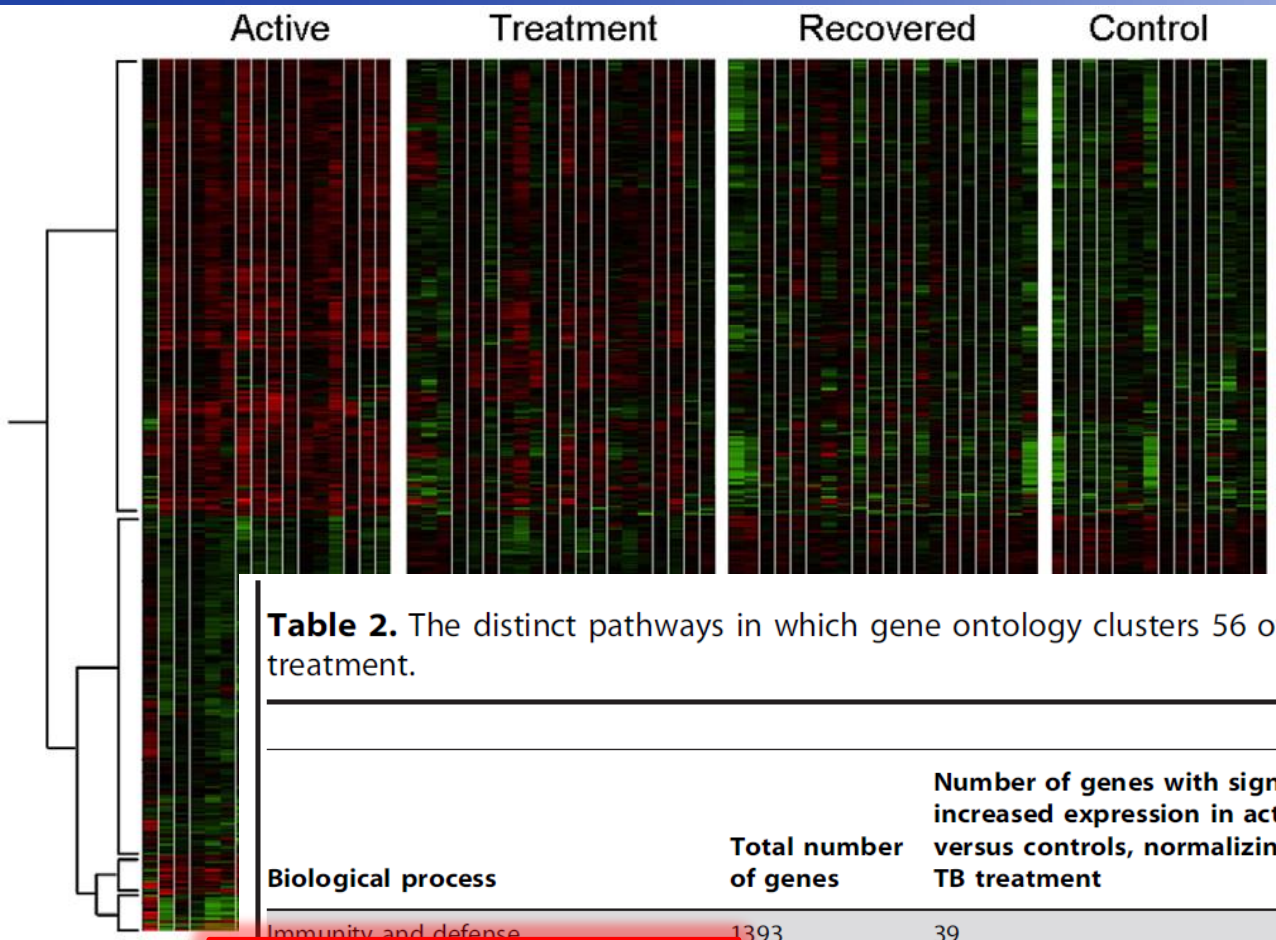
Gene ID	Fold change
drotrecogin alpha	
TFPI	1.74
SERPINB2	1.61
CP	1.52
GGCX	1.49
SERPIND1	1.58
SERPINB6	1.82
SERPINE1	1.43
THBD	0.53
F5	0.48
Vasopressin	
GNG11	1.73
GNG5	1.43
GNAQ	0.58
Hydrocortisone	
ALOX5	0.34
ANXA1	0.64
Norepinephrine	
NNMT	1.32
MOXD1	1.42

Fold change based on significance analysis of microarrays (SAM), with q-value for each gene listed equal to 0. Values shown represent expression ratio of type 2 relative to type 1.

McConnell KW, Crit Care 2010

Maslove et al, Crit Care 2012

Transcriptional Profiling of Host response TBC patients



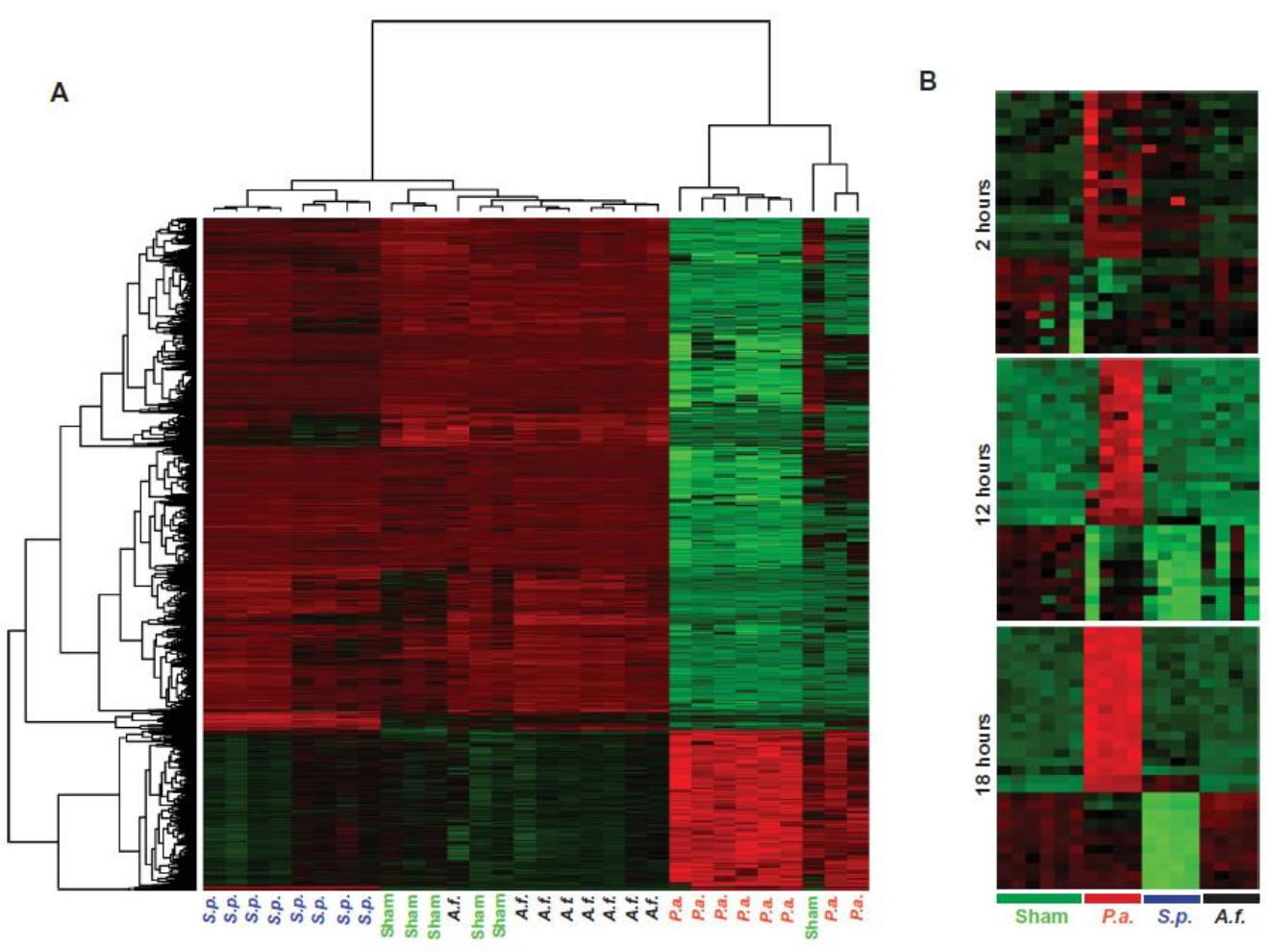
- GEP discriminates phases of TBC treatment in patients

Table 2. The distinct pathways in which gene ontology clusters 56 of the genes with increased expression normalizing during treatment.

Biological process	Total number of genes	Number of genes with significantly increased expression in active TB patients versus controls, normalizing during TB treatment	Expected number of genes	Bonferroni corrected p-value
Immunity and defense	1393	39	11.20	1.83×10^{-10}
Type-I interferon mediated immunity	69	10	0.55	5.21×10^{-8}
Macrophage mediated immunity	150	7	1.21	0.034

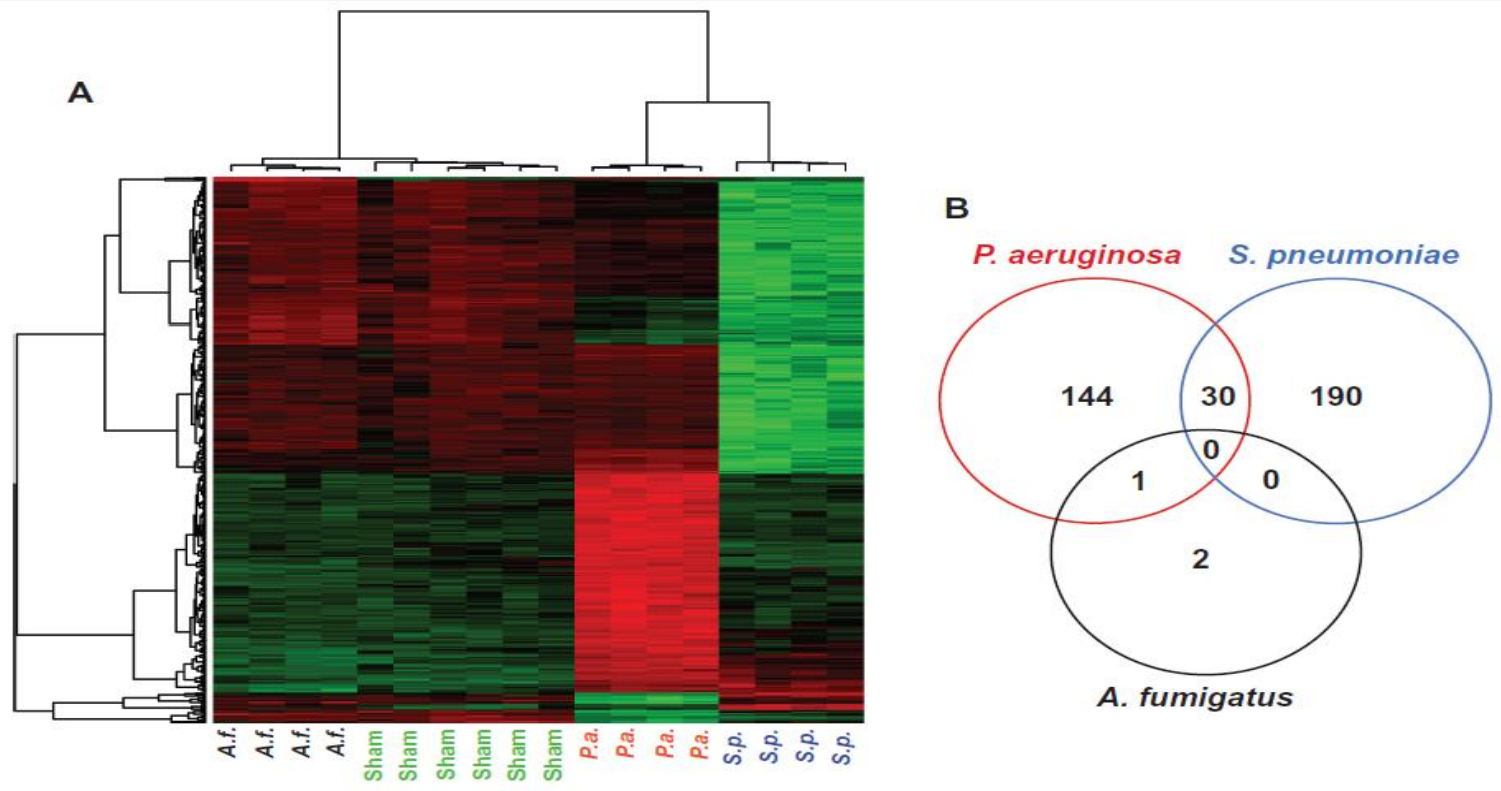
Ottenhoff THM, PLOS One 2012

Transcriptional profiling of GEP for inhalative pathogens



Evans, Resp Res 2010

Transcriptional profiling of GEP for inhalative pathogens – Specific signature for *A. fumigatus*



- Preclinical data from murine models suggest a specific gene signature for Aspergillus detected from homogenized lung tissues

Evans, Resp Res 2010

Transcriptional profiling of GEP for IA – Clinical Data

ICAAC 2012

52nd ICAAC – Sept. 9-12 - *San Francisco*

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Session: 190-Clinical Mycology I

Tuesday, Sep 11, 2012, 11:15 AM - 1:15 PM

Presentation Title: M-1695 - Correlation of Circulating Human Blood RNA Biomarkers and Clinical Outcomes in an Observational Invasive Aspergillosis (IA) Trial

Abstract:

Background: Developing IA therapies is hampered by difficulty in assessing early treatment response and clinical prognosis. A biomarker interposed between treatment initiation and patient outcome will aid therapy evaluation. **Methods:** We examined human blood gene expression level correlation with clinical outcomes (exploratory objective) in a post-hoc assessment of a 12-week prospective, multi-center, observational clinical study (clinicaltrials.gov NCT00854607 primary objective fungal serum sugar correlation). 116 patients ≥16 years of age with presumptive possible, probable, or proven IA infection were enrolled and started on antifungal therapy. Those with proven or probable IA after 14 days were assessed for clinical outcomes (complete or partial response, or failure) at weeks 6 and 12 (n = 51). Blood was drawn for mRNA expression profiling at four time points across a 12-week period: baseline, weeks 2, 6, and 12. **Results:** T and NK cell activation pathway genes (e.g. ZAP70, NFAT) were differentially expressed over time (outcomes correlation p=0.01). ANOVA on outcomes and time points with transcript expression data normalized to pretreatment identified a gene panel with 158 differentially expressed genes (outcomes correlation p = 1e-08). **Conclusions:** RNA expression profiling appears promising as a biomarker of anti-fungal drug clinical response. Prospective evaluation and validation are warranted.

Host Response Profiling in IA – Our Approach

- Accumulation of GEP Signatures of proven/probable IA patients from Blood and BAL
- Assessment of feasibility of performing host GEP Analysis on BAL
- Identification of a specific gene expression signature for patients with proven/probable IA as opposed to other infectious agents
- Clinical trial will hopefully start sometime in the future (given financing)

Host Response Profiling in IA – Open Questions ?

- Analysis feasible in leukopenic patients or patients under therapeutic immunosuppression ?
- GEP Analysis feasible in BAL given the lack of standardized BAL volume ?
- Should an enriched cell population be assessed for GEP (e.g. macrophages, T-cells ?
- GEP analysis probably best in biopsy samples, yet rarely obtained in the hematologic patient population