APPLICATION NOTE

Reveal Sources of Immune Modulation with the Single-Cell Innate Immune Solution

Bruker's Single-Cell Innate Immune solution is helping researchers detect functional cellular differences that correlate to immune modulation.

In this Application Note we outline:

- Why understanding single-cell function is critical
- Understanding the role of innate immune cells in immune suppression
- How single-cell innate immune data differentiates functional response to TLR2 stimulation of MS patients vs. controls
- The importance of monocyte/macrophage cellular polyfunctionality
- How the Innate Immune solution reveals monocyte response to pathogenic ligands

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Multiplexed Proteomic Characterization: Filling the Existing Gap in Full Cellular Characterization from Single-Cells

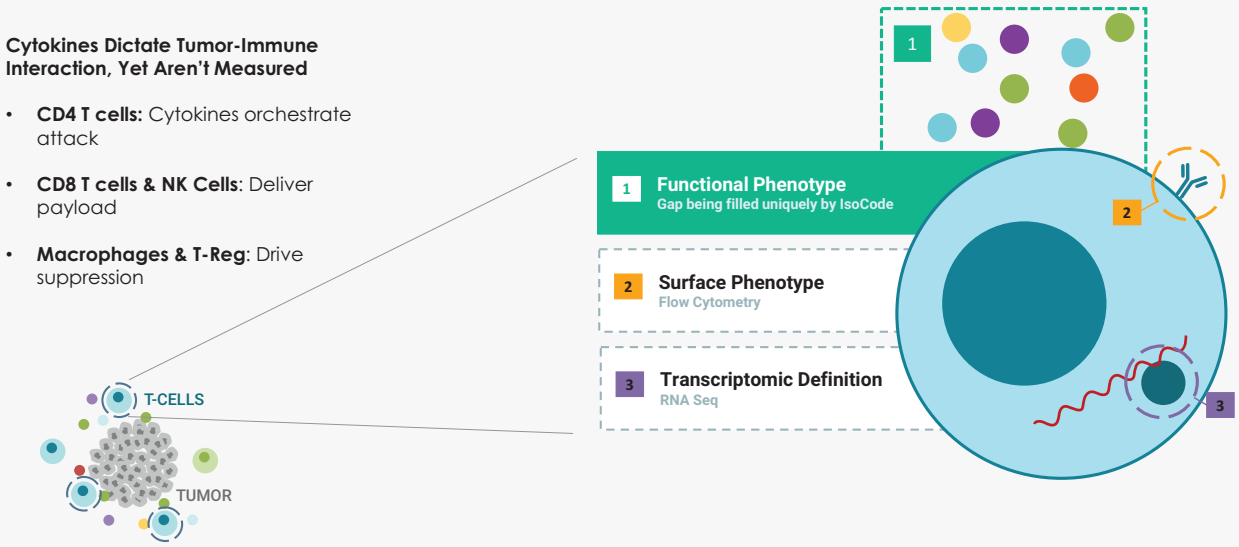

Figure 1 | Through analysis of cellular RNA or surface phenotypes alone, functional extracellular phenotypic differences that reveal the biological drivers of patient response may be missed.

Why Understanding Single-Cell Function is Critical

Bruker has developed the only technology able to phenotype each immune cell by its extracellular function, termed "functional phenotyping." Through functional phenotyping, Bruker is answering critical challenges by functionally defining each T cell, monocyte, and NK cell, providing researchers with data on exactly what each immune cell functionally produces with a range of 30+ cytokines.

Each immune cell is different, thus the complete characterization of each individual cell is critical.

While transcriptome is measured via RNA seq, and surface phenotype is measured via flow cytometry, the complete cellular definition is incomplete without measuring extracellular cytokines that are doing the work in tumor immunology.

For single-cells, Bruker is filling a gap as the only technology to do something very, obviously critical — directly detecting the functional cytokines that matter.

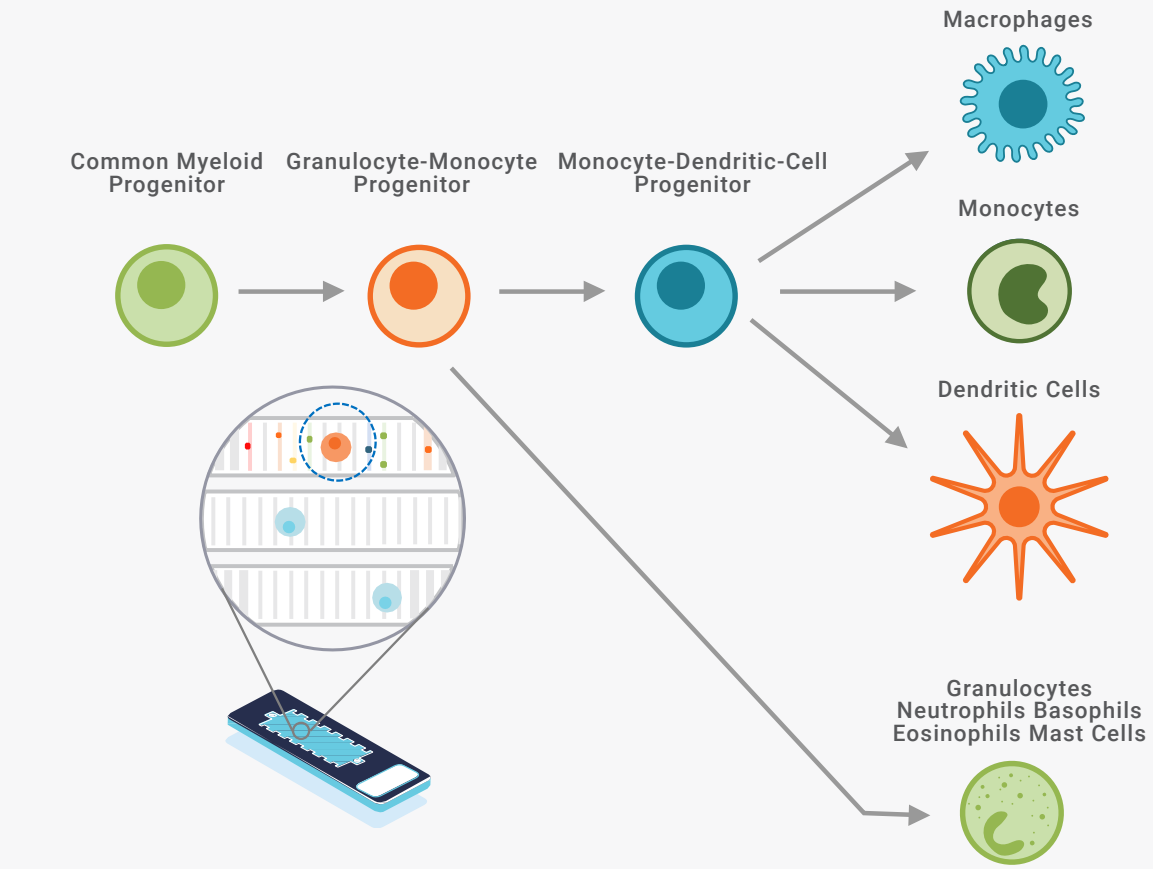

Figure 2 | Myeloid cells provide key aspects of both orchestrating the attack of and suppression of the immune system towards the tumor, analyzed on the Single-Cell Innate Immune solution (adapted from [1]).

Understanding the Role of Innate and Myeloid Cells in Immune Suppression

In cancer immunology, T-cells and NK cells have historically been the most discussed, but myeloid cells provide key aspects of both orchestrating the attack of and suppression of the immune system towards the tumor. The IsoLight is unlocking the Extracellular Functional Phenotype of each of these myeloid cells with the Single-Cell Innate Immune solution.

Cells from the myeloid lineage including cell types such as, Neutrophils, MDSCs, and Monocytes, together compose a critical arm of the immune system, largely responsible for innate defense against an array of pathogens (Figure 2). As an example, monocytes utilize the range of extracellular function to destroy invasive cells.

Upregulated monocyte activity has been shown in various studies to dampen anti-tumor response, leading to poorer prognosis in patients.

These myeloid cell types are now being assayed using the Single-Cell Innate Immune solution in a variety of research areas including: cancer immunology, cell & gene therapy, inflammation, cancer signaling pathways, and infectious disease & vaccines.

Prep, Run, Analyze

The IsoLight Proteomic Hub

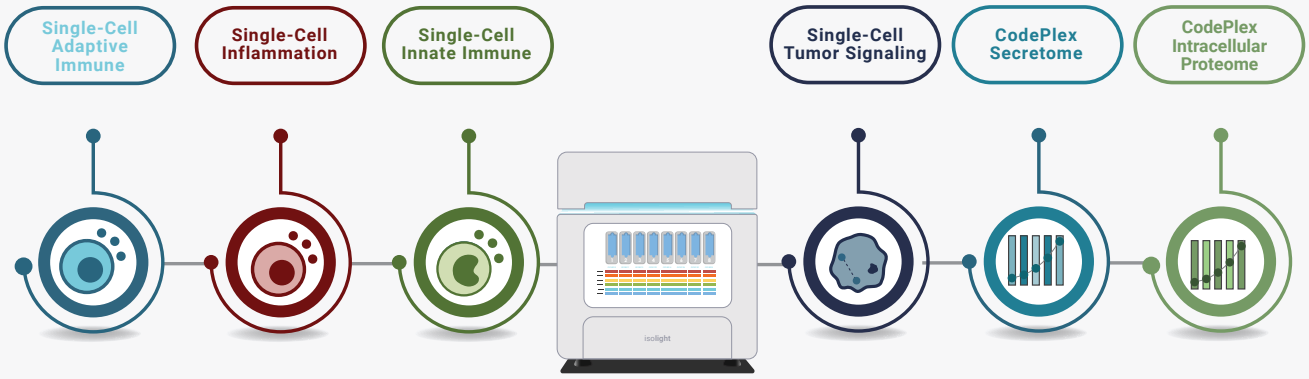

Figure 3 | The IsoLight system serves as a proteomic hub, enabling both single-cell and population cytokine assays for a variety of cell types on a single system.

Bruker's Single-Cell Innate Immune solution application

Bruker's Single-Cell Innate Immune solution is helping researchers detect functional cellular differences that correlate to immune suppression. Translating this single-cell innate and myeloid data to uncover insights enables advancement of discovery and development programs. The Single-Cell Innate Immune solution does this by revealing the sources of cellular differences based on the ability to detect what each immune cell is truly secreted in a highly multiplexed manner.

The IsoLight Proteomic Hub (Figure 3) connects over 30 cytokines that orchestrate the immune response from each innate and myeloid cell, back to the cell itself (Figure 4). The Single-Cell Innate Immune solution detects the subsets of polyfunctional immune cells secreting multiple cytokines that correlate to immune suppression, which traditional technologies would have missed.

The Single-Cell Innate Immune Panel

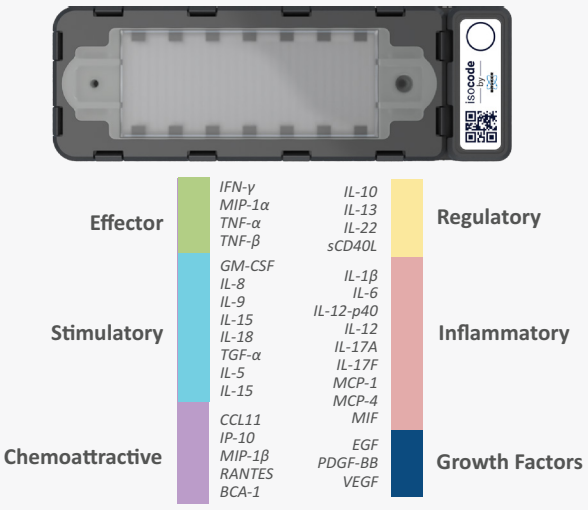

Figure 4 | Bruker's Single-Cell Innate Immune Panel measures over 30 cytokines that orchestrate the immune response from each innate and myeloid cell, back to the cell itself.

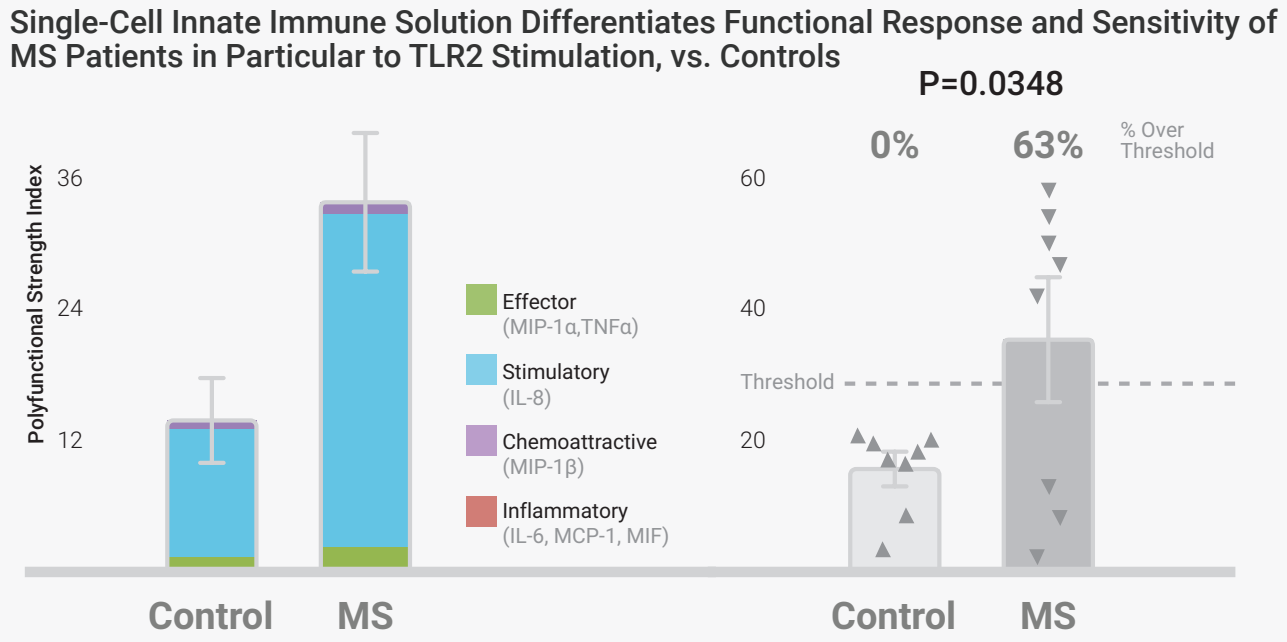

Figure 5 | Identification of enhanced MS response to TLR2 stimulation. CD14+ monocyte PSI was calculated for control (n=8) and MS patient samples (n=8) stimulated for 24 hours with P3C (TLR2 stimulation). (a) The PSI of the MS patients samples was notably higher than in the control samples; the PSI of both sample groups was dominated by polyfunctional IL-8 secretions. (b) Statistical analysis showed that the PSI of the MS patients (mean = 34) are statistically higher (p = 0.0348) than the PSI of the control samples (mean = 14). The level of PSI representing the upper threshold of control responses (based on the control IQR) is depicted by the dashed line. The percent of responses above threshold is depicted for controls and total MS patients [2]. The difference in PSI between the two groups suggests an important role of TLR2 signaling in mediating MS disease progress driven by monocytes.

Single-Cell Innate Immune Data Differentiates Functional Response to TLR2 Stimulation of MS Patients vs. Controls

Here we demonstrate how Bruker's systems can be used to help segment the heterogeneity of monocytes, and find particular monocyte subsets which secrete diverse combinations of cytokine functions that play a role in autoimmunity, specifically MS [2]. We used the 32- plex Single-Cell solution to simultaneously detect the full spectrum of secreted cytokines associated with innate immunity, facilitating comprehensive dissection of the functional states of each single-cell within the total cell population.

Most investigations into the role of innate immunity in MS have focused on response to toll-like receptor 4 (TLR4). However, using Bruker's systems, we saw a large fraction of MS patients exhibiting enhanced responsiveness to TLR2 stimulation of CD14+ monocytes by Pam3CSK4 (P3C) relative to the control population. Specifically, 62.5% of MS patients exhibited PSI values above the interquartile range (IQR)-derived upper threshold of responses, while none of the control responses were above this threshold.

The major difference between MS patients and controls

was the higher percentage of monocytes secreting two or more cytokines among the MS cohort (Figure 5). In this study, we were also able to show that the enhanced response in MS patients is specific to TLR2 stimulation by P3C and not evident via stimulation of TLR4 on monocytes by lipopolysaccharide (LPS). While there is some increase in TLR4 responsiveness in this MS cohort, we observed a more notable enhancement in response to TLR2 stimulation. Additional evaluation of this dataset allowed us to stratify the MS cohort into patients with relapsing-remitting MS (RRMS) and patients with progressive MS. One of two progressive MS patients showed enhanced response whereas four of the six RRMS patients showed enhanced response. These results confirmed data generated independently (discussed in [2]) and suggest that the highest frequency of enhanced TLR2 responders within the MS cohort may be found among the patients with progressive forms of the diseases. The ability to stratify MS patients based on monocyte functionality may be especially powerful given the observed differences in therapeutic response for those with the different forms of the disease.

Importance of Monocyte/Macrophage Cellular Polyfunctionality

Here we show how Bruker's Single-Cell Innate Immune solution were used to identify functional immune cell subsets which correlated with *in vivo* activity. In a study published in *Blood Cancer Journal* [3], CD14+SIRPα^{hi} (signal regulatory protein-α) monocytes/macrophages were associated with an inferior survival in Follicular Lymphoma (FL), and increased numbers of the CD14−SIRPα^{low} subset appeared to correlate with a better survival. The Single-Cell Innate Immune solution revealed an inhibitory mechanism of enhanced IL-10 in single-cell polyfunctional CD14+ SIRPα^{hi} subsets, reducing T cell proliferation, which provides the functional insights of Mo/MΦs subset for FL patients, which were implicated in inferior survival of patients. Cellular IL-10 level showed the critical mechanistic importance of monocyte function in the interaction with T cell functional response, which correlated with patients who had inferior survival.

Cytokine/chemokine Production by CD14+SIRPα^{hi}, CD14−SIRPα^{low}, or CD14−SIRPα^{neg} cells

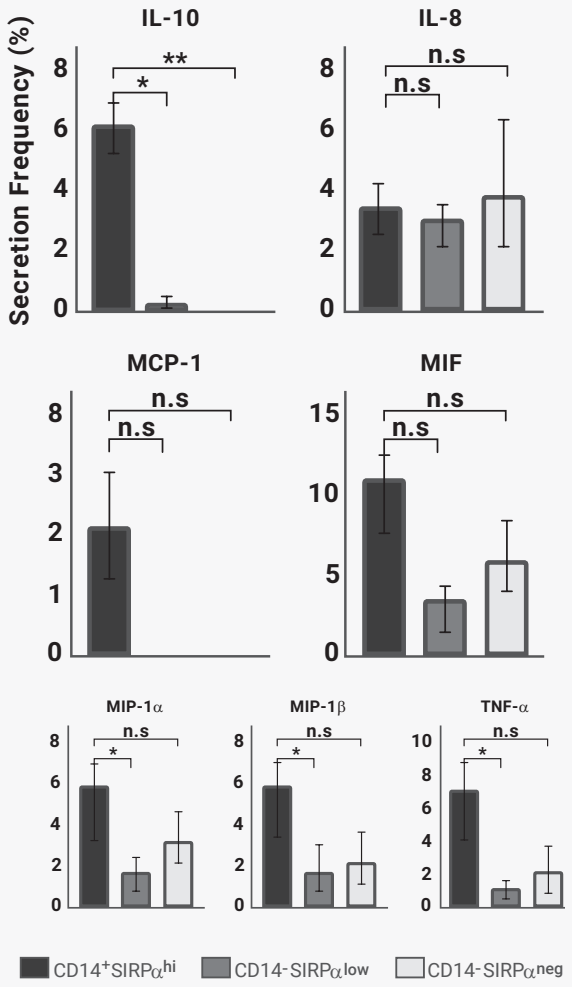

Figure 6 | Cytokine/chemokine production by CD14+SIRPα^{hi}, CD14−SIRPα^{low}, or CD14−SIRPα^{neg} cells determined by the the Single-Cell Innate Immune solution. Results were expressed as the percentage of cells producing cytokines/chemokines. *p < 0.05; **p < 0.01; n.s. no significant difference. N = 3.[3].

Monocyte Response to Pathogenic Ligands

In a study published in PNAS, researchers employed the Innate Immune solution to profile differentiated macrophages after stimulation with lipopolysaccharide (LPS), the ligand of toll-like receptor 4 (TLR4) [4]. These profiles revealed a level of functional heterogeneity and pathogenic activation that was previously uncharacterized.

By functionally phenotyping single-cells before and after LPS stimulation, it was revealed that macrophage inhibitory factor (MIF) enabled the activation of LPS-induced cytokine production. Bruker technology enables a more complete analysis of immune function in response to pathogenic stimulation, revealing heterogeneity in cell populations that are phenotypically similar.

Advanced cellular visualizations and mapping revealed five distinct subpopulations (Figure 7). Notably, stimulation with LPS increased the polyfunctional subpopulation. Analysis of stimulation with additional ligands, PAM3, and poly(I:C), also affected the proportions of cells in each of the subpopulations. It was determined that LPS induced a more potent response than either PAM3 or poly(I:C), though each resulted in upregulation in different cytokines.

The results show that in response to TLR ligands, heterogenous population structures are highly conserved. Taken as a whole, it is clear that phenotypically similar cell populations are potentially functionally and behaviorally heterogenous, further showing the need for functional characterization at the single-cell level for additional detail and accuracy when analyzing immune activation in response to pathogens.

Functional Heterogeneity and Subpopulations of U937-Derived Macrophages in Response to TLR Ligands

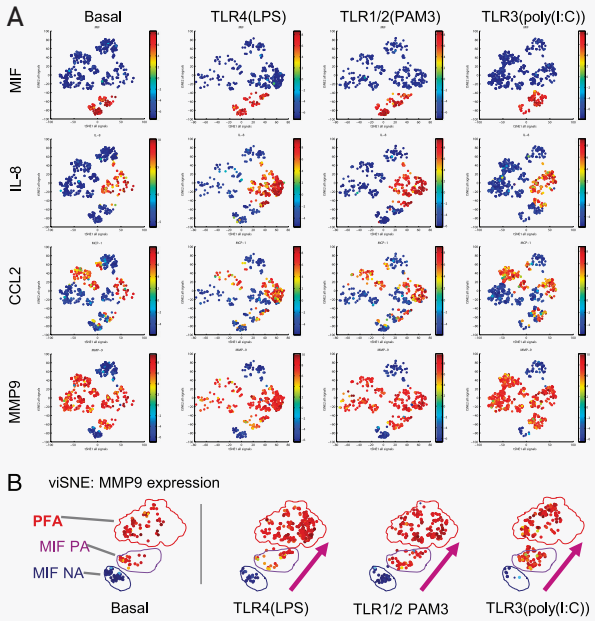

Figure 7 | Profiling of the same single-cells before and after LPS stimulation identified a role for macrophage inhibitory factor (MIF) to potentiate the activation of LPS-induced cytokine production.

Conclusion

- The complete cellular definition is incomplete without measuring the extracellular cytokines that are doing the work in tumor immunology.
- The Single-Cell Innate Immune solution provides insight into how myeloid cells orchestrate the attack and suppression of the immune system towards the tumor.
- The Single-Cell Innate Immune solution differentiates functional response and sensitivity of MS patients in particular to TLR2 stimulation, vs. controls.
- Key biomarkers revealed by the Single-Cell Innate Immune solution showed the critical mechanistic importance of monocyte function in the interaction with T cell functional response, which correlated with patients who had inferior survival in Follicular Lymphoma.
- Insights revealed by the Innate Immune solution reveal heterogeneity in phenotypically similar cell populations in response to pathogenic ligands

References

1. Ransohoff, R., Cardona, A. The myeloid cells of the central nervous system parenchyma. Nature 468, 253–262 (2010)
2. Fujiwara M, Anstadt EJ, Flynn B, Morse K, Ng C, Paczkowski P, et al. Enhanced TLR2 responses in multiple sclerosis. Clin Exp Immunol., 193(3):313-326 (2018). PMCID: PMC6150258
3. Chen, Y., Kim, H.J., Wu, H. et al. SIRPα expression delineates subsets of intratumoral monocyte/macrophages with different functional and prognostic impact in follicular lymphoma. Blood Cancer J. 9, 84 (2019)
4. Lu Y, Xue Q, Eiselle M R, Sulistijo E S, Brower K, Han L, Amir E D, Pe'er D, Miller-Jensen K, Fan R. Highly multiplexed profiling of single-cell effector functions reveals deep functional heterogeneity in response to pathogenic ligands. Proceedings of the National Academy of Sciences Feb 2015, 112 (7) E607-E615;