Research Highlights

Highlights from the latest articles in biomarkers in medicine

An IL-17-mediated paracrine network promotes tumor resistance to antiangiogenic therapy

Evaluation of: Chung AS, Wu X, Zhuang G *et al.* A newly identified mechanism of resistance to anti-angiogenic therapy. *Nat. Med.* 19(9), 1114–1123 (2013).

Antiangiogenic therapy has improved cancer treatment successes, but there are still cases of resistance to this therapy. Understanding inherent or acquired resistance mechanism(s) to therapy is always an important step in the search for new treatments. Drug resistance can come from the tumor itself (which typically takes time) or it can come from the environment of the tumor; for example, fibroblasts, immune cells or the extracellular matrix (which appears more rapidly). This article displays the series of experiments describing a fascinating mechanism involving Th17 cells and IL-17, which are likely to lead to treatment improvement.

To try to understand the mechanism, the authors used two cell lines, one resistant (EL4) and the other sensitive (Tib-6) to antiangiogenic therapy. They assayed a series of potential perpetrating secreted mediators. The most important one was IL-17, known to be secreted by Th17 cells. IL-17 can then influence the production of other proinflammatory factors such as G-CSF, IL-6 and Bv8.

In another series of experiments, implanting EL4 tumors in mice, they showed that anti-IL17 antibodies can improve the response to antiangiogenic treatment. As EL4 cells have no IL-17 receptors, they also concluded that the effect may come from a paracrine (and not autocrine) function.

Furthermore, the authors demonstrated that the increase in IL-17 induced an increase in G-CSF. This increase in

G-CSF induced the mobilization and recruitment of immature myeloid cells (CD11b⁺Gr1⁺ cells) in the tumor micro-environment. These cells were also shown to be functionally modulated by IL-17.

Secreted proteins were shown to mainly come from the stroma (and not from the tumor). The responsible cells were identified as tumor-associated fibroblasts. Data have shown that IL-17 can induce tumor-associated fibroblasts to produce pro-inflammatory cytokines such as G-CSF through MAPK and NF-κB signaling.

IL-17 is produced by Th17 cells. The authors compared the Lewis lung carcinoma tumor burden between mice with and without IL-17 receptors (IL-17RC knockout). The burden was decreased in the IL-17RC knockout mice. Likewise, concomitant treatment by an anti-IL17 antibody improved the effect of the anti-angiogenic treatment in a lung orthotopic model in wild-type mice.

The data in this paper show how adaptive immunity could be involved in the resistance to antiangiogenic therapy. It also shows that IL-17 is not only responsible for cell recruitment, but can also influence stromal cell functions.

The tumor types should also be kept in mind. The data above are applicable to lung and colon cancer, where it is known that Th17 cell presence in tumors correlates with bad prognosis. Different interpretation is to be made in the cases of ovarian and gastric cancers.

These data would support giving special attention to Th17 cell presence in tumor biopsies depending on the tumor types: another step towards personalized healthcare to improve the treatment of cancer patients.

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How to link biomarkers using physiologically based pharmacokinetic models

Evaluation of: Tsamandouras N, Rostami-Hodjegan A, Aarons L. Combining the 'bottom-up' and 'top-down' approaches in pharmacokinetic modelling: fitting PBPK models to observed clinical data. *Br. J. Clin. Pharmacol.* doi:10.1111/bcp.12234 (2013) (Epub ahead of print).

Translational medicine aims at describing mechanistic links between biomarkers, from *in vitro* ones to clinical end points. The integration and articulation of those biomarkers are typically organized by modeling and simulation techniques. In this article, the authors delineate the process by which they can combine different approaches of modeling, using the specific example of pharmacokinetics.

The article summarizes well the three levels of complexity of modeling techniques. The first level is the empirical approach; that is, data are described by a mathematical model chosen on the best fit. The second level is best described by being between level 1 and 3; that is, it is a so-called semi-mechanistic model using in some parts an empirical approach and in other parts a mechanistic approach. Level 3 is the mechanistic model, and in pharmacokinetics, it is the complex physiologically based pharmacokinetic (PBPK) model. In this case, all key pathways through which a test drug is expected to pass in a living organism, are computed.

Recent tendency in drug development is to favor the use of level 3. This was made possible by improvements in computers and software by the increased use of *in silico* methods and by the development of *in vitro* and *in vivo* extrapolation methods.

Numerical sensitivity of a model is often assessed by sensitivity analysis. This type of analysis attempts to evaluate the origin of the variability of the model output by assessing different sources of variation included in the model. Ultimately, there

is the good advice that all PBPK models should be assessed on the plausibility of the physiological parameters and their assigned range of values.

Correlation between parameters must be carefully examined. If there is a high correlation, one of the parameters should be quantified using a physiologically plausible value.

For parameter estimation, two aspects are underlined. The authors suggest that most of the parameters should be fixed from physiological values and only a few should be estimated by model optimization techniques. In addition, the reliability of the estimate should be described, and this is often a challenging task for nonphysiologically parameterized values.

A very interesting clarification is made between the two terms uncertainty and variability. "Variability refers to differences attributable to environmental or genetic factors," and "Uncertainty is variation that derives from errors in the experimental procedure, measurement, modelling and assumptions of the studied system."

The possibility of incorporating Bayesian statistics approaches with PBPK is discussed. One key advantage is to bring a distribution of probability perspective; that is, a sort of variability description. However, a disadvantage is the need for high-power, high-speed computers and highly (or at least specifically) skilled scientists.

Ultimately, good PBPK models are sometimes considered overparameterized because they include many different parameters. For a whole-body system, the number of dimensions to analyze can be limited by the size of the datasets. It is then reasonable to use techniques such as lumping procedures and semi-mechanistic models instead.

The conclusion is driven by common sense and proposes "to develop mechanistically sound models with clinical relevance." In drug development, mechanistic approaches are often key to resolving difficult hurdles because they permit the identification of the key parameter(s) driving the failure of a particular molecule.

A new biomarker to identify high-risk renal transplant patients

Evaluation of: Loupy A, Lefaucheur C, Vernerey D *et al.* Complement-binding anti-HLA antibodies and kidney-allograft survival. *N. Engl. J. Med.* 369(13), 1215–1226 (2013).

Organ transplantation has had a superb success story but there are still patients who reject their graft. It is critical to improve the *a priori* detection of those patients so that antirejection therapy is closely monitored. It is already known that anti-HLA antibodies can provoke rejection, in particular in kidney transplantation. The mechanism of rejection involves activation of the complement cascade. Anti-HLA antibodies can be characterized by their C1q (one of the complement system proteins) binding properties. Small exploratory studies were performed supporting the concept that complement binding of anti-HLA antibodies could be responsible for allograft rejection.

In this paper, the authors report the results of a large study in 1016 kidney allograft patients. The objective of the study was to assess whether the presence of C1q-binding donor-specific anti-HLA antibodies after transplantation could evaluate the risk of kidney rejection.

Patients were enrolled from two kidney transplantation centers in Paris (France) between January 2005 and January 2011. There was also an external validation cohort of similar patients from a third center. Acute clinical rejection was defined according to the international Banff classification criteria. Immunosuppression protocols and treatment of rejection were similar in the three centers.

Graft biopsies were obtained from 845 patients free from rejection and 171 patients with acute clinical rejection in the first year post-transplantation. The biopsy specimens were scored and graded following the Banff criteria.

All patients had blood samples assayed for circulating donor-specific anti-HLA

antibodies. Those patients positive for the anti-HLA antibodies were also assayed for the presence of C1q binding.

Out of the 1016 kidney transplant patients, three groups were identified after transplantation based on the presence or absence of anti-HLA antibodies and the presence or absence of C1q-binding properties: 700 had no circulating anti-HLA antibodies (anti-HLA negative group), 239 had noncomplement binding anti-HLA antibodies (anti-HLA positive group) and 77 had complement-binding anti-HLA antibodies (C1q positive group).

A total of 1 year after transplantation, acute rejection occurred in 171 patients: 96 underwent T-cell-mediated rejection (56%) and 75 underwent antibody-mediated rejection (44%).

T-cell-mediated rejection occurred in 18% of the C1q positive group (14 out of 77), in 13% of the anti-HLA positive group (30 out of 239) and in 7% of the anti-HLA negative group (52 out of 700; $p < 0.001$).

Antibody-mediated rejection occurred in 48% of the C1q positive group (37 out of 77) and in 16% of the anti-HLA positive group (38 out of 239; $p < 0.001$).

The C1q positive group had more widespread and severe histologic lesions.

The 5-year graft survival after transplantation was 54% in the C1q positive group, 93% in the anti-HLA positive group and 94% in the anti-HLA negative group ($p < 0.001$).

The external validation group of 643 patients showed the same results.

The presence of C1q-binding donor-specific anti-HLA antibodies is an independent predictor of renal allograft loss in the 5 years after transplantation. The involvement of anti-HLA antibodies in graft rejection was already known, but the C1q-binding detection was not sensitive enough until now. These findings might also be relevant for other transplant organs.

There might also be a therapeutic interest in finding ways to block the C1q activation pathway in those patients.