50 INVITED

Genetic aberrations as determinants of response to chemotherapy

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Background: The vast majority of clinical trials combining chemotherapy plus targeted agents are based on empirical criteria and in general yield short-lived responses and no substantial benefit in prolonging survival in a meaningful way for the patient. Moreover, some chemotherapy drugs may well be antagonistic when combined with others and even with targeted drugs. Therefore, the first endeavor should focus on the correct choice of chemotherapy drugs and combinations. Experimental evidence suggests that BRCA1 overexpression enhances sensitivity to docetaxel and resistance to cisplatin. RAP80 and Abraxas are interacting proteins that form complexes with BRCA1 and could modulate the effect of BRCA1. In order to further examine the effect of EGFR mutations and BRCA1 mRNA levels on outcome in advanced NSCLC, we performed a prospective non-randomized phase II clinical trial, testing the hypothesis that customized therapy would confer improved outcome over non-customized therapy.
Material and Methods: We treated 123 metastatic non-squamous cell lung carcinoma patients using a customized approach. Patients with EGFR mutations received erlotinib, and those without EGFR mutations received chemotherapy with or without cisplatin based on their BRCA1 mRNA levels: low, cisplatin plus gemcitabine; intermediate, cisplatin plus docetaxel; high, docetaxel alone. An exploratory analysis examined RAP80 and Abraxas expression.
Results: Median survival exceeded 28 months for 12 patients with EGFR mutations, and was 11 months for 38 patients with low BRCA1, 9 months for 40 patients with intermediate BRCA1, and 11 months for 33 patients with high BRCA1. Two-year survival was 73.3%, 41.2%, 15.6% and 0%, respectively. Median survival was influenced by RAP80 expression in the three BRCA1 groups. For example, for patients with both low BRCA1 and low RAP80, median survival exceeded 26 months. RAP80 was a significant factor for survival in patients treated according to BRCA1 levels (hazard ratio, 1.3 [95% CI, 1−1.7]; P = 0.05).
Conclusions: Chemotherapy customized according to BRCA1 expression levels can optimize survival, and RAP80 could play a crucial modulating effect on this model of customized chemotherapy.

Scientific Symposium (Mon, 21 Sep, 16:15−18:15)
Prostate cancer

53 INVITED

Advances in molecular understanding of prostate cancer

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Our understanding of the molecular basis of prostate cancer increased significantly over the past decade. As early as 1990 the first papers on specific genetic changes in prostate cancer appeared. Subsequent molecular genetic studies confirmed the frequent involvement of genetic changes on chromosome 13q, 8p, 16 q and others. Subsequently several specific genes were identified that were mutated, deleted or amplified, and genes with a know etiological role in carcinogenesis gained particular interest, such as oncogenes and tumor suppressor genes. The p53 gene was mutated in a relatively small fraction of prostate tumors. Another tumor suppressor gene, however, appeared to be more commonly deleted and/or mutated in prostate cancer, PTEN. More recently, it was shown that in the majority of prostate cancers the erg gene is activated by fusion an androgen regulated gene, TMPRSS2. The biological relevance of both changes was supported by germ line activation (erg) or inactivation (PTEN), suggesting that these genes are critically associated with prostate cancer development. The most common genetic change in patients that aren't responsive to the currently registered endocrine therapies (CRPC) is amplification of the androgen receptor, suggesting an important role of the AR in progression to CRPC. In fact, these insights provide targets for improved diagnosis, prognosis and therapy of prostate cancer. The molecular adaptation that leads to the intracrine production of androgens provided the rationale basis for next generation endocrine therapies.
Through the emergence of high throughput 'omics' platforms the discovery rate of molecular targets/pathways went up significantly and apart from the 'classical' genes, recurrent epigenomic changes and changes in non coding – and microRNAs are identified. Collectively, these findings should be the basis of identifying the specific molecular fingerprint of individual cancers for which molecular profile specific, rather than disease specific, therapies should be indicated.

54 INVITED

Prostate cancer: the bone paradigm . . .

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Prostate cancer (PCa) is the most common cancer and the third leading cause of cancer death among men in developed countries. Prostate cancer cells spreading out of the prostate are characterized by an exquisite tropism to bone. In men progressing under hormonal therapy, bone is indeed the primary metastatic site in 80% of the patients. Later on, in end-stage disease, 90% of the patients or more will have bone metastases. Bone metastases can alter the physiological bone remodeling processes and invade the surrounding structures. This results in a morbid cortege of complications such as pathological fractures, pain, spinal chord compression and anemia, best known as skeletal-related event (SRE). In addition, bone metastases and SRE occurs nowadays mostly in an osteopenic environment resulting from the chronic used of androgen deprivation therapy (ADT) as a mainstay treatment of advanced and metastatic disease. Testosterone, indeed, is critical for the normal bone physiology, and ADT causes rapid and profound bone loss, a process named CTIBL, or Cancer Treatment Induced Bone-Loss.
This unique interaction between a selective osteotropism of prostate cancer and a profound alteration of the bone composition creates an interesting "bone paradigm" that we will need to tackle.
Several issues will be addressed:

- There is a crucial need for developing imaging technologies to correctly address drug efficacy at the level of bone metastases. TC-99m bone scan is not imaging the cancer itself but the bone remodeling that goes with it and therefore is not adequate to measure tumor response in bone. New technologies based on MRI and/or PET technologies are currently developed.
- The intense basic research activity on the pathophysiology of bone disease has increased our level of understanding on the "vicious circle" of bone metastases and help indentifying the key molecules that are driving the development of bone metastases such as PTHrP, RANK/RANK-L/OPG, and ET-1/ET-A pathways.
- New drugs are on their way through Phase III trials, giving us the ability to specifically interfere with the progression and maybe the development of bone metastases.

55 INVITED

Drug resistance in metastatic prostate cancer

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Most men who develop metastatic prostate cancer respond initially to androgen deprivation therapy (ADT) and the preferred initial treatment is with a long-acting GnRH agonist, with a peripheral anti-androgen given transiently to prevent flare. The duration of response is variable with a median of ~2 years. About one third of patients then respond to addition of a peripheral anti-androgen such as bicalutamide, and of those who respond a smaller proportion may respond subsequently to its withdrawal. Previously most men were then regarded as hormone-resistant, although some would respond successively to further hormonal manoeuvres such as estrogens, dexamethasone or ketoconazole plus hydrocortisone. The term castration-resistant prostate cancer (CRPC) is more appropriately used to describe the disease at this stage. Studies have shown that androgen-dependent pathways remain active within CRPC, despite very low levels of circulating androgens, and are often associated with intra-tumour androgens and/or increased androgen receptor expression. This has led to new approaches to overcome or delay resistance to ADT that include use of intermittent treatment, and new drugs such as abiraterone acetate, a potent and specific inhibitor of CYP17, an enzyme that catalyses two steps in androgen synthesis, and MDV 3100, a more potent and structurally different inhibitor of the androgen receptor than bicalutamide. The status of these strategies will be reviewed. However, there is also evidence for a population of tumour-initiating (stem) cells within prostate cancer that do not express the androgen receptor, perhaps setting limits on efficacy of all forms of ADT.
For men who are no longer responding to ADT, chemotherapy with docetaxel has been shown to provide a modest improvement in survival

(median ~3 months), and in symptoms and quality of life. All men develop resistance to docetaxel, although some may respond to further courses of docetaxel after an interval off treatment. Thus mechanisms of resistance are likely to include unstable (epigenetic) changes in cells as well as more stable genetic changes such as over-expression of drug export proteins or mutations in genes encoding microtubule-associated proteins. Docetaxel has been given with a variety of other agents in attempts to increase efficacy or delay the onset of resistance, but thus far none has been successful. Likewise administration of other chemotherapeutic drugs after progression on docetaxel, including mitoxantrone, epothilones and satraplatin has shown some but limited benefit, consistent with the presence of common mechanisms of resistance to different types of chemotherapy.

We, and others, have shown that some anticancer drugs have limited distribution from blood vessels within solid tumours, and poor drug access is an as yet unexplored cause of drug resistance. We have also shown that accelerating repopulation of surviving tumour cells may occur between cycles of chemotherapy, leading to tumour regrowth even if there is no change in intrinsic sensitivity to the drugs used. Administration of cytostatic molecular targeted agents between doses of cycle-active docetaxel to inhibit tumour cell repopulation, and the use of new generation hypoxia-selective agents used in combination with conventional chemotherapy to complement limited drug distribution, are two promising approaches that will be investigated in clinical trials.

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Resistance mechanisms to EGFR and HER2 inhibitors and strategies to overcome them

57 INVITED

A functional genetic approach identifies the PI3K pathway as a major determinant of Trastuzumab resistance in breast cancer

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Background: At present, 1 year of Trastuzumab (Herceptin®) treatment has become part of the adjuvant treatment of women with early stage HER2-positive breast cancer. However, it is still largely unclear why almost half of the breast cancer patients that over-express HER2 is non-responsive to Trastuzumab based therapy or become resistant to Trastuzumab during treatment. Therefore, clinical questions such as how to optimize patient selection or prevent resistance to Trastuzumab-based therapy still await answers.

We present here a method to identify biomarkers associated with non-responsiveness to Trastuzumab in cell culture and demonstrate that these biomarkers have predictive value in a patient cohort treated with Trastuzumab combination therapy.

Material and Methods: As an unbiased approach to identify genes involved in Trastuzumab resistance, we used a large-scale RNA interference genetic screen in the HER2-overexpressing breast cancer cell line BT-474. We have generated a library of 24,000 shRNA retroviral vectors targeting some 8,000 human genes for suppression by RNA interference and developed a technology to rapidly screen such libraries, named siRNA bar code screening.

Results: Of the 8,000 genes tested, we found that only knock down of PTEN conferred resistance to Trastuzumab. Decreased PTEN expression results in hyper activation of the PI3K pathway. Significantly, activating mutations in the gene encoding the p110a catalytic subunit of PI3K (PIK3CA) have been identified in some 25% of primary breast cancers potentially mimicking the effects of PTEN loss. Indeed, overexpression of the breast cancer-derived mutant PIK3CA (H1047R) also conferred resistance to Trastuzumab in cell culture. These findings are consistent with a major role of the PI3K pathway in the development of resistance to Trastuzumab.

Our cell culture experiments led us to investigate whether PI3K pathway activation is able to predict Trastuzumab resistance in the clinic. In a cohort of 55 patients treated for metastatic breast cancer, activation of the PI3K pathway, as judged by the presence of oncogenic PIK3CA mutations or low PTEN expression, was associated with poor prognosis after Trastuzumab therapy. Furthermore, the combined analysis of PTEN and PIK3CA identified twice as many patients at increased risk for progression compared to PTEN alone.

Conclusions: The present work highlights the central importance of PI3K signalling in risk for progression after Trastuzumab-based therapy, which in turn suggests combination therapeutic strategies to treat Trastuzumab unresponsive breast cancer or to prevent emergence of resistance. We are currently analyzing a cohort of 50 patients who received neo-adjuvant Trastuzumab-based therapy to test whether PI3K pathway activation status validates as a biomarker for response prediction in the neo-adjuvant setting.

58 INVITED

Inhibition of IGF signalling as cancer therapy

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Inhibition of growth factor signalling is emerging as one of the most promising therapies for cancer treatment. Many new approaches to disrupting the insulin-like growth factor (IGF) system have been developed and early clinical trials demonstrate promising results. The IGF system is composed of an interlinked network of ligands and receptors functioning in endocrine, autocrine, and paracrine pathways. As with any endocrine system, there are multiple ligands and receptors. Insulin, IGF-I, and IGF-II interact with the type I IGF receptor (IGF1R), insulin receptor (InsR), and hybrid receptors composed of subunits of both IGF1R and InsR. Preclinical data suggest that in order to predict the physiologic consequences of IGF1R inhibition on the cancer phenotype, a more complete understanding of other growth regulatory pathways needs to be defined. For example, IGF stimulation of cancer cells may result in enhanced cell motility and augment metastatic potential. In other cells, IGF may enhance proliferation and survival. These cancer phenotypes are regulated not by the receptor, but by activation of signalling pathways downstream of IGF1R. Since some of the phenotypes, such as inhibition of metastasis, are not easily observable in clinical trials, attention to these downstream signalling events may help develop predictive biomarkers for appropriate selection of patients. Our preclinical data suggest that the insulin receptor substrate (IRS) adaptor proteins are required regulators of IGF1R regulated biology. Moreover, gene expression signatures downstream of these adaptor proteins differ. IGF1R signalling also cooperates with other known growth regulatory pathways such as EGFR/HER2, estrogen receptor, and integrins. As new anti-IGF therapies emerge, attention to downstream signalling events needs careful consideration in order to identify predictive biomarkers and to define successful combination therapies.

59 INVITED

Understanding the genetic basis of resistance to EGFR targeted therapies to personalize colorectal cancer treatment

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Personalized cancer medicine based on the genetic milieu of individual colorectal tumors has long been postulated but until recently this concept was not supported by clinical evidence. The advent of the EGFR-targeted monoclonal antibodies cetuximab and panitumumab has paved the way to the individualized treatment of metastatic colorectal cancer (mCRC). There is clear evidence that mCRCs respond differently to EGFR-targeted agents and that the tumor specific response has a genetic basis. From the initial observation that cetuximab or panitumumab as monotherapy are effective only in 10−20%, of mCRCs, knowledge has being gained on the molecular mechanisms underlying primary resistance to these agents. The role of oncogenic activation of EGFR downstream effectors such as KRAS, BRAF, PIK3CA and PTEN on response to therapy will be discussed. The rapid and effective translation of these findings into predictive biomarkers to couple EGFR-targeted antibodies to the patients that benefit from them will be presented as a paradigm of modern clinical oncology. Unresolved questions such as understanding the molecular basis of response as well the mechanisms of secondary resistance will be discussed as the future fundamental goals in this research field.