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Concluding remarks and future perspectives
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CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The survival rates of patients with metastatic colorectal cancer have improved significantly due to the recent introduction of novel therapies. Moreover, the use of cetuximab, panitumumab and bevacizumab has accelerated the implementation of molecular testing in colon cancer diagnostics. Indeed, KRAS mutation detection in stage IV colorectal cancer has become standard practice in many pathology laboratories and other markers like BRAF V600E and PIK3CA will probably follow in the near future. However, molecular characterization is currently used only in stage IV disease and not in earlier disease stages. Therefore, stage II and III are less well characterized at the molecular level, forming a rather heterogeneous disease group. Several parameters such as tumor localization, mismatch repair (MMR) status or tumor histology influence clinical behavior but are often not taken into account when defining clinical subsets. Hence, this intertumor heterogeneity, together with intratumor heterogeneity and tumor plasticity are probably reasons for the discrete improvements in survival rates in these stages¹ and the somewhat disappointing results of some of these novel clinical trials of the last decade²⁴.

In stage II and III colon cancer, the identification of patients at risk of relapse, due to therapy resistance or to tumor intrinsic aggressiveness, is needed in order to improve disease management and outcome. Therefore, the main focus of this thesis was to identify molecular prognostic and predictive markers of response to therapy in stage II and III sporadic colon cancer. Predictive markers can identify patients who are not likely to respond to a certain chemotherapeutic drug, helping to decrease unnecessary exposure to that particular drug and thus toxicity. On the other hand, prognostic markers will identify patients with a poor natural history of their disease who will probably benefit from adjuvant chemotherapy or even from a more aggressive form of therapy than recommended by the guidelines.
Pharmacogenetics & Predictive Markers

Since the mid-nineties the therapy guidelines for colon cancer management recommend the use of adjuvant chemotherapy after curative intended surgery for all patients with stage III colon cancer. This recommendation improved colon cancer patients’ survival. Risk of cancer related death in stage III patients was reduced in 29% (CI 13-42%) with 5-FU monotherapy. Combination of 5-FU with oxaliplatin, administered since 2005, reduced the risk of cancer related death with another 20%. Despite this significant improvement in patient survival, a large percentage of patients apparently still do not experience any benefit from the treatment.

We studied eight polymorphisms in genes coding for proteins involved in the metabolism of 5-FU and oxaliplatin such as the thymidylate synthase (TYMS), thymidine phosphorylase (TYMP), dehydrogenase dehydroxilase (DYM), orotate phosphoribosyltransferase (OPRT), glutathion S transferase Pi (GSTPI), excision repair cross complementing group 1 (ERCC1) and excision repair cross complementing group 2 (ERCC2) genes in stage III sporadic colon cancer patients. None of the polymorphisms studied was found to be a reliable marker predictive of therapy response in stage III disease.

These markers have been extensively studied by us and others, not only at the DNA level and in colon cancer but also at expression level (mRNA and protein) and in other types of cancer. Their value as predictive markers remains elusive because of conflicting results. However, research groups did find certain genotypes (alone or combined) of the cited genes predictive of therapy response in colon cancer patients or indicative of therapy toxicity. The contradictory and inconclusive results might be explained by the retrospective character of the majority of the studies and the diversity of molecular techniques used. Furthermore different SNPs and genotype combinations were tested. On top of this, the results of functional experiments assessing the effect of a certain SNP in protein function and expression turned out to be contradictory as well.

This all makes the biological interpretation of the results complicated and probably unreliable. Moreover, most of the studies examined a heterogeneous population of patients including different disease stages, and differently located cancers (left-, right-sided or rectum). All these factors might give rise to the different results. Finally, even studies reporting positive relations between certain genotypes and disease outcome or therapy toxicity,
advocate for validation in prospective trials or larger cohorts before implementation in clinical practice. Therefore, based on the existing literature and our experience, we conclude that in order to discard or implement such genetic markers in clinical practice, two types of studies are mandatory. Firstly, functional studies reporting the effect of SNPs on gene expression, protein function etc. are essential to determine which SNPs are likely to be relevant in pharmacogenetics. Secondly well-designed association studies, within prospective clinical trials are needed. Prospective clinical trials fulfill several criteria like large cohorts of patients that are carefully documented and homogeneously treated. Indeed, this approach has been used for reporting associations between toxicity and SNPs but less frequently for therapy response. Another possibility is a retrospective study with an exploratory and a validation cohort. However, to study therapy response and toxicity, patients should have been equally treated and clinical course should have been carefully documented. In case of an exploratory and a validation cohort these are frequently not equally treated because of differences in disease management depending on location and time of diagnosis.

Tan et al recently published the results of a clinical trial with rectal carcinoma patients. These patients were randomized between standard 5-FU based chemoradiotherapy and alternative 5-FU combined with irinotecan chemoradiation, on the base of a TYMS genotype. The authors concluded that classification of patients based on their genotype and subsequent variation of the therapy was feasible and that therapy results improved with this pharmacogenetic approach. The latter trial constitutes a first step towards the incorporation of molecular pharmacogenomic testing in personalizing therapies in early stages of colon cancer. However, it also raises the question whether there is enough scientific evidence for these kinds of trials.
Somatic Mutations and Prognostic Markers

Given the enormous expansion of targeted therapies and their price coming with it, prognostic/predictive markers are essential for accurate patient’s classification and disease management. In addition, the molecular classification of patients and their tumors will contribute to more homogeneous study groups increasing the probability of reliable results and improvements in colon cancer therapy.

Prognostic markers are useful for a more accurate classification of patients and can identify different prognostic subgroups as seen for the $BRAF$ V600E mutation. The latter mutation not only identifies patients with a poor prognosis independently of disease stage and even MMR status, but it also seems to characterize a type of tumor with an own genomic profile that is different than double wild type tumors.

However, not all mutations have such a clear association with prognosis like $BRAF$ V600E. We show in this thesis that the mutation in exon 20 of the $PIK3CA$ gene has only prognostic value in stage III disease and not in stage II. Moreover, we also report that gene-gene interactions can affect the prognostic effects of certain makers. This is the case of $TP53$ inactivation which prognostic effects are greatly affected by the differential expression of the $CSNK1A1$ gene. Thus, although very complex, gene-gene interactions also need to be studied within the scope of prognostic markers research.

In conclusion, molecular analysis of cancer cells can potentially aid to classify tumors more accurately and to manage patients accordingly. However, prognostic biomarkers need to be integrally analyzed to be able to explore genetic interactions and subtle molecular relations. Therefore, combined genetic, genomic, epigenetic and expression studies should be carried out. Likewise, basic functional research is essential to learn more about genetic interactions and to be able to correctly interpret data obtained from new techniques like SNP arrays or next generation DNA/RNA sequencing.
Future Perspectives

To decrease colorectal cancer death in the future, two complementary approaches are necessary; on one hand, disease prevention and early diagnosis and on the other hand accurate disease classification should be established for personalized therapy.

Disease prevention

By implementing screening programs for colorectal cancer, malignant tumor development can be prevented by excising premalignant polyps and cancer can be diagnosed at earlier stages like stage I/II when surgery is still curative. Indeed, several Western countries are implementing population based screening programs. The expectation, in The Netherlands, is to reduce colorectal cancer incidence and prevent mortality in 2400 patients per year out of the current 10 000 and therefore reduce treatment costs75.

Molecular disease classification

The second approach consists of the identification, validation and general implementation of molecular signatures identifying colon cancer subgroups. At this moment, all colon cancer patients with stage III and high risk stage II are treated equally without taking into account tumor molecular signatures. Recently, two colon cancer gene expression signatures associated with disease recurrence and poor prognosis in early stages have been published76,77. Although they have not been approved for clinical use yet, they represent one step forward in the use of molecular profiling in colon cancer classification.

In the near future standard molecular stratification of patients and tumors should be able to define subgroups of patients leading to personalized treatment protocols. A problem herewith is intratumor heterogeneity as well as tumor plasticity. Intratumor heterogeneity has been recognized for a long time now by surgeons, oncologists, pathologists and molecular biologists. Tumors may contain multiple clones that do not necessarily share the same molecular signatures or phenotypes. The different clones in a particular tumor evolve in time depending on tumor environmental influences like growth factors, hypoxia, inflammation, immune responses, stroma composition, et cetera. The study of these topics is technically challenging and difficult to solve and these subjects are therefore underrepresented in the literature78,79.
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With the introduction of targeted therapies in cancer management tumor heterogeneity and plasticity have become even more important. These therapies target strategically chosen genes with activating mutations, based on the so-called oncogene addiction model. According to this model, cancer cells become dependent of certain activating mutations in key molecules in cell division, cell survival and signaling pathways. Cancer cells can circumvent the blocking of signaling pathway by acquiring novel mutations or switching to other pathways, thereby becoming resistant to a particular therapy. This adaptive capacity of the tumor is probably responsible for the relatively rapid relapses after treatment with targeted therapies seen in clinical practice. Moreover, it is currently unknown what is the minimum percentage of resistant or sensitive cells in order to consider a tumor resistant or sensitive for a given therapy. Thus, the clinical consequences of intratumor heterogeneity need to be further investigated as it is now technically more feasible.

Molecular pathology enabling the molecular classification of tumors and molecular biomarker determination in cancer diagnostics already plays an important role in daily clinical oncologic practice. However, put into perspective, a relatively very small proportion of molecular markers makes it eventually to daily clinical practice. In the nearby future and derived from the use of new technologies, molecular diagnostics will probably play an essential role in tumor classification. Therefore, specific training of future pathologists in the field of molecular diagnostics is pivotal in order to ensure an effective interplay between oncologists, pathologists and molecular biologists, leading to patient tailored therapy.

Besides, a vivid debate is taking place in the Netherlands about the implementation of molecular diagnostics in pathology laboratories. At the present time, it is not legally regulated which laboratory can carry out molecular diagnostics; both academic and non academic centers perform molecular diagnostics in pathology. However, the level of complexity is rapidly increasing, the development of new tests is expensive and specific expertise and knowledge are mandatory to interpret results. Thus, to ensure high quality, competitive prices and ongoing technological research and innovation, expertise and technologies should be, in our opinion centralized.

Molecular prognostic markers or molecular tumor signatures will aid to classify colon cancer patients more accurately in order to improve disease management and patient outcome. These molecular signatures could be a complement to decision making tools for chemotherapy choice and even improve these tools. Molecular predictive
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Markers will help reduce cancer treatment toxicity of unnecessary therapy regimens. Collaborative studies to reach enough statistical power are mandatory to identify small subgroups of patients behaving differently clinically. Integral typing of these samples i.e. at a genetic, genomic, regulatory, epigenetic and expression level, mRNA, miRNA and protein levels, is recommended. Basic functional research is mandatory to make biological sense of data obtained from whole genome analyses. Finally, elucidating the role of intratumor heterogeneity and plasticity is an important challenge to understand tumor biology and really accomplish personalized therapy in the future.
References


38. Ichikawa W, Uetake H, Shirota Y, et al: Combination of dihydropyrimidine
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63. Villafranca E, Okruzhnov y, Dominguez MA, et al: Polymorphisms of the repeated
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78. Marusyk A, Polyak K: Tumor heterogeneity: causes and consequences. Biochim Biophys Acta 1805:105-17


