Part I  Introduction

Minimal Residual Disease and Circulating Tumor Cells in Breast Cancer: Open Questions for Research .......................... 3

1 How can the Study of MRD and CTCs Help Us to Better Understand Breast Cancer Metastasis? ............................................. 4

2 Is There any Preferred Technology for CTC Detection and Characterization? ................................................................. 4

3 What is the Role of Other Blood-Based Biomarkers like Circulating Enothelial Cells and Circulating Nucleic Acids? ............ 4

4 Should DTC/CTC Detection and Characterization be Used in Current Clinical Practice? .................................................. 5

5 What are the Challenges in Drug and CTC Co-Development? .................................................. 5

6 Future Perspectives .................................................. 5

References ........................................................................ 6

Part II  Minimal Residual Disease and Breast Cancer Metastasis

Self-Seeding in Cancer .................................................. 13

1 Introduction .................................................. 14

2 Self-Seeding Model of Malignant Growth: The Biological Basis for Self-Seeding .................................................. 14

3 Mathematical Foundation of Self-Seeding .................................................. 15

4 Prevailing Mysteries: Unpredictable Metastatic Pathways ........ 16

4.1 Why do Some Patients Without Axillary Nodal Involvement Still Develop Systemic Metastases? And Why do Some Patients With Axillary Nodal Metastases not Develop Metastases Elsewhere, Even If Those Nodal Metastases are not Removed by Surgery or Irradiated? .................................................. 16

4.2 Why is it That so Few Patients Present With Gross Metastatic Disease, Even When They May Have Large Untreated Tumors for a Long Time? .................................................. 18
5 Molecular and Genetic Implications of Self-Seeding ....... 19
  5.1 Why is DCIS so Molecularly and Genetically Similar to Invasive Cancer? .......................... 19
  5.2 Why Does Sampling a Random Tiny Portion of a Tumor Reflect the Behavior of the Larger Tumor? ............ 19
  5.3 Why is Mammographic Breast Density a Risk Factor for Breast Cancer? .................................. 19
6 Clinical Applications of Self-Seeding .......................... 20
7 Conclusion ...................................................... 20
References ....................................................... 21

Microenvironments Dictating Tumor Cell Dormancy .......... 25
1 Introduction .................................................... 26
2 Theoretical Considerations and Evidence for the Potential Scenarios of Tumor Dormancy ...................... 29
  2.1 Scenario 1: The Target Organ Microenvironment as a Determinant of DTC Dormancy .................. 29
  2.2 Scenario 2: Primary Tumor “Stress Microenvironments” Determine DTC Fate ......................... 31
  2.3 Scenario 3: Early Dissemination as a Determinant of DTC Dormancy .................................. 33
3 Conclusions and Perspectives ................................. 36
References ........................................................ 37

Part III Technologies for Circulating Tumor Cell and Disseminated Tumor Cell Detection and Characterization

Immunomagnetic Separation Technologies .......................... 43
1 Introduction ...................................................... 44
2 CTC Enrichment and Detection Methods .......................... 44
3 Detection of CTCs by Flow Cytometry without Prior Enrichment ....................................... 46
4 Immunomagnetic CTC Enrichment .................................. 48
5 Detection of Immunomagnetically Enriched CTCs by Flow Cytometry ..................................... 50
6 Detection of Immunomagnetically Enriched CTCs by Microscopy ................................... 52
References ........................................................ 54

Microfluidic Technologies .......................................... 59
1 Introduction ...................................................... 60
2 Physical Separation Methods ...................................... 62
3 Affinity-Based Separation Methods ................................. 64
4 Conclusions and Future Outlook ................................. 65
References ........................................................ 66
## EPISPOT Assay: Detection of Viable DTCs/CTCs in Solid Tumor Patients

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>70</td>
</tr>
<tr>
<td>2 Epispot Procedure</td>
<td>70</td>
</tr>
<tr>
<td>3 CTC Enrichment Step</td>
<td>71</td>
</tr>
<tr>
<td>4 Detection of Viable and not Apoptotic DTCs/CTCs</td>
<td>72</td>
</tr>
<tr>
<td>5 Clinical and Translational Leads</td>
<td>73</td>
</tr>
<tr>
<td>6 Challenges and Future Directions</td>
<td>74</td>
</tr>
<tr>
<td>References</td>
<td>75</td>
</tr>
</tbody>
</table>

## Advances in Optical Technologies for Rare Cell Detection and Characterization

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>78</td>
</tr>
<tr>
<td>2 Optical Detection Systems</td>
<td>79</td>
</tr>
<tr>
<td>2.1 Flow Cytometry</td>
<td>79</td>
</tr>
<tr>
<td>2.2 Laser Scanning Cytometry</td>
<td>80</td>
</tr>
<tr>
<td>2.3 Automated Digital Microscopy</td>
<td>80</td>
</tr>
<tr>
<td>2.4 Optical Enrichment Using Laser Scanning</td>
<td>80</td>
</tr>
<tr>
<td>3 Discussion</td>
<td>83</td>
</tr>
<tr>
<td>References</td>
<td>83</td>
</tr>
</tbody>
</table>

## Size-Based Enrichment Technologies for CTC Detection and Characterization

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>88</td>
</tr>
<tr>
<td>2 Size-Based Isolation of CTCs by Microfiltration</td>
<td>88</td>
</tr>
<tr>
<td>3 Emerging Technologies for Size-Based Enrichment of CTCs</td>
<td>92</td>
</tr>
<tr>
<td>4 Affinity-Based Versus Size-Based Methods for CTC Enrichment</td>
<td>93</td>
</tr>
<tr>
<td>5 Conclusion</td>
<td>94</td>
</tr>
<tr>
<td>References</td>
<td>94</td>
</tr>
</tbody>
</table>

## Emerging Technologies for CTC Detection Based on Depletion of Normal Cells

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTC Identification Relies on its Separation Strategy</td>
<td>98</td>
</tr>
<tr>
<td>2 Enrichment Methodologies</td>
<td>99</td>
</tr>
<tr>
<td>3 Advantages of CTC Pre-Enrichment by Depletion of Normal Cells (Negative Depletion)</td>
<td>99</td>
</tr>
<tr>
<td>4 Depletion of Normal Cells Prior to Flow Cytometry or Other Optical Analyses</td>
<td>100</td>
</tr>
<tr>
<td>5 Magnetic Depletion Technologies</td>
<td>101</td>
</tr>
<tr>
<td>6 Clinical Results With CTCs or Cancer Associated Cells Identified With Negative Depletion</td>
<td>102</td>
</tr>
<tr>
<td>6.1 Breast Cancer</td>
<td>102</td>
</tr>
<tr>
<td>7 Squamous Cell Carcinoma of the Head and Neck</td>
<td>105</td>
</tr>
<tr>
<td>8 Epithelial Mesenchymal Transition</td>
<td>105</td>
</tr>
</tbody>
</table>
Molecular Assays for the Detection and Characterization of CTCs

1 Introduction. .................................................. 112
2 Molecular Detection Technologies for CTC Analysis .................................. 112
   2.1 RT-qPCR .................................................... 114
   2.2 Multiplex RT-qPCR ....................................... 114
   2.3 Liquid Bead Array ....................................... 115
3 Molecular Characterization of CTCs ........................................... 115
   3.1 Gene Expression Studies ................................ 115
4 Quality Control in CTC Detection Systems: Comparison of Different Methodologies ........................................ 118
5 Conclusions: Future Perspectives ........................................... 119
References .................................................. 120

Multiplex Molecular Analysis of CTCs

1 Introduction. .................................................. 126
2 CTC Enrichment Using the MagSweeper Technology ............................... 126
3 Sensitive Nucleic Acid and Protein Isolation Techniques .......................... 127
4 Pre-amplification Methods ....................................... 128
5 Estimating the Contribution by Remaining Leukocytes ................................ 129
6 Selection of CTC-Specific Molecular Markers ...................................... 129
7 Data Analysis and Validation ....................................... 131
8 Examples of Multiplex and Other Molecular Analyses of CTCs .................. 132
9 Additional Examples of Multiplex Analysis of CTCs ................................ 138
10 Concluding Remarks ........................................... 138
References .................................................. 138

Part IV Other Blood-Based Biomarkers

Circulating DNA and Next-Generation Sequencing ..................................... 143
1 Introduction. .................................................. 144
2 Circulating DNA in Cancer ........................................... 144
3 Next-Generation Sequencing of Solid Tumours ....................................... 146
4 Tumour-Specific Rearrangements in ctDNA ........................................... 147
References .................................................. 148

Circulating MicroRNAs as Noninvasive Biomarkers in Breast Cancer ............. 151
1 Introduction. .................................................. 152
2 Circulating MicroRNAs in Breast Cancer ........................................... 153
3 Stability and Origin of Circulating MicroRNAs ...................................... 154
4 Conclusions ................................................................. 157
References ................................................................. 158

Circulating Endothelial Cells and Circulating
Endothelial Progenitors ................................................. 163
1 Introduction ............................................................. 164
2 CEC and CEP Phenotypes .......................................... 164
3 CEC Kinetic and Cancer Treatment .............................. 165
4 CEP Role in Cancer Growth and Metastasis Development .... 166
5 Conclusions ............................................................. 167
References ................................................................. 168

Part V  Disseminated Tumor Cells and Circulating Tumor Cells
in Breast Cancer Clinical Research and Practice

DTCs in Breast Cancer: Clinical Research and Practice ......... 173
References ................................................................. 177

CTCs in Primary Breast Cancer (I) .................................. 179
1 Introduction ............................................................. 180
2 CTC Detection by Immunocytochemistry ....................... 180
3 Prevalence and Prognostic Relevance of CTCs in the Context
of Neoadjuvant Treatment ............................................. 181
4 Prognostic Value of CTCs Before Adjuvant Chemotherapy .... 182
5 CTC Evaluation Immediately After Chemotherapy
and During Recurrence-Free Follow-Up ........................... 183
6 Conclusions and Therapeutic Implications ..................... 183
References ................................................................. 184

CTCs in Primary Breast Cancer (II) ................................. 187
1 Introduction ............................................................. 188
2 CTC Detection by RT-PCR for CK19 mRNA .................... 188
3 Prognostic Value Before Adjuvant Chemotherapy ............. 188
4 Prognostic Value After Adjuvant Chemotherapy ............... 189
5 Prognostic Value in Molecular Subtypes ......................... 190
6 Prognostic Value During Hormonotherapy and Follow-Up .... 190
7 Conclusions ............................................................. 191
References ................................................................. 192

CTCs in Metastatic Breast Cancer ..................................... 193
1 Introduction ............................................................. 194
2 CellSearch® System for CTC Identification in MBC .......... 195
3 Clinical Implication of CTC Molecular Characterization .... 197
References ................................................................. 199
HER2-Positive DTCs/CTCs in Breast Cancer .............................. 203
1 Introduction .............................................................. 204
2 Evaluation of the HER2 Status of DTCs/CTCs .................... 205
3 Clinical Impact of HER2-Positive DTCs/CTCs in Primary
  Breast Cancer ............................................................. 206
4 Clinical Impact of HER2 Positive DTCs/CTCs in Metastatic
  Breast Cancer ............................................................. 208
5 Conclusion and Perspectives ........................................... 210
References ................................................................. 210

DTCs/CTCs in Breast Cancer: Five Decades Later .................... 217
1 Metastatic Breast Cancer ................................................ 218
2 Early Stage Breast Cancer .............................................. 221
3 Recommendations ........................................................ 223
References ................................................................. 223

Part VI Drug and Circulating Tumor Cell Co-development

Challenges in Drug and Biomarker Co-Development .................. 229
1 Introduction .............................................................. 230
2 When Should Co-Development Be Considered? ..................... 231
3 General Issues to Be Considered in Co-Development ............... 231
4 Assay Development in the Context of Co-Development:
  Planning for Success ...................................................... 233
5 Regulatory Challenges in Co-Development ........................... 235
6 Conclusions .............................................................. 236
References ................................................................. 237

Challenges and Opportunities in the Use of CTCs for Companion
Diagnostic Development .................................................... 241
1 Introduction ............................................................. 242
2 On the Relevance of CTCs as a Source of Representative
  Cancer Tissue for Predictive Dx ...................................... 242
3 Technologies for Molecular Characterization of CTCs ............ 243
4 HER2 Diagnostics in CTCs ............................................. 246
5 Applications of Predictive Biomarker Analyses in CTCs
  to Treatment of Metastatic and Early Stage Disease .............. 247
6 The Path Forward ........................................................ 248
7 Conclusions .............................................................. 249
References ................................................................. 250
Minimal Residual Disease and Circulating Tumor Cells in Breast Cancer
Ignatiadis, M.; Sotiriou, C.; Pantel, K. (Eds.)
2012, XIV, 254 p., Hardcover
ISBN: 978-3-642-28159-4