
Contents

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 Endothelial 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.	69
1 Introduction.	70
2 Epispot Procedure	70
3 CTC Enrichment Step	71
4 Detection of Viable and not Apoptotic DTCs/CTCs.	72
5 Clinical and Translational Leads	73
6 Challenges and Future Directions.	74
References	75
 Advances in Optical Technologies for Rare Cell Detection and Characterization.	77
1 Introduction.	78
2 Optical Detection Systems	79
2.1 Flow Cytometry	79
2.2 Laser Scanning Cytometry	80
2.3 Automated Digital Microscopy	80
2.4 Optical Enrichment Using Laser Scanning	80
3 Discussion.	83
References	83
 Size-Based Enrichment Technologies for CTC Detection and Characterization.	87
1 Introduction.	88
2 Size-Based Isolation of CTCs by Microfiltration	88
3 Emerging Technologies for Size-Based Enrichment of CTCs	92
4 Affinity-Based Versus Size-Based Methods for CTC Enrichment	93
5 Conclusion	94
References	94
 Emerging Technologies for CTC Detection Based on Depletion of Normal Cells.	97
1 CTC Identification Relies on its Separation Strategy	98
2 Enrichment Methodologies	99
3 Advantages of CTC Pre-Enrichment by Depletion of Normal Cells (Negative Depletion)	99
4 Depletion of Normal Cells Prior to Flow Cytometry or Other Optical Analyses.	100
5 Magnetic Depletion Technologies	101
6 Clinical Results With CTCs or Cancer Associated Cells Identified With Negative Depletion	102
6.1 Breast Cancer	102
7 Squamous Cell Carcinoma of the Head and Neck	105
8 Epithelial Mesenchymal Transition	105

9	Current Assumptions about “Normal Cell”	107
	References	107
Molecular Assays for the Detection and Characterization of CTCs . . .		111
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.		125
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