Contents

List of Contributors  XI
Preface   XV

1  Biomarkers – Past and Future  1
Siegfried Neumann
1.1 Introduction  1
1.2 Definitions of Biomarkers  2
1.3 Biomarkers in the Past  3
1.4 Novel Molecules and Structural Classes of Biomarkers by New Technologies  7
1.5 Biomarkers in Drug Research  9
1.6 Current Development and Future Trends for Biomarkers in Laboratory Diagnostics  12
1.6.1 Biomarker Test Validation  12
1.6.2 Companion Diagnostics in Clinical Pharmacology  14
1.6.3 Biomarker Multivariate Index Assays  16
1.6.4 Regulatory Policies on Biomarker Tests  17
1.7 Summary and Outlook  19
References  20

2  Quantitative Proteomics Techniques in Biomarker Discovery  23
Thilo Bracht, Dominik Andre Megger, Wael Naboulsi, Corinna Henkel, and Barbara Sitek
2.1 Introduction  23
2.1.1 General Considerations  24
2.2 2D-Difference Gel Electrophoresis  27
2.3 Mass Spectrometry-Based Proteomics  29
2.3.1 Principles and Instrumentation  29
2.3.1.1 Ionization Methods  29
2.3.1.2 Mass Analyzers  30
2.3.2 Label-Free Protein Quantification  30
2.3.2.1 Area Under Curve (AUC) or Signal Intensity Measurement  30
2.3.2.2 Spectral Counting  31
3 Biomarker Qualification: A Company Point of View 39
Maximilian Breitner, Kaidre Bendjama, and Hüseyin Firat

3.1 Introduction 39
3.2 Biomarker Uses 40
3.3 Biomarker Types 41
3.4 Validation vs. Qualification 43
3.5 Strategic Choices in Business Models 43
3.6 Validation of Analytical Methods 44
3.6.1 Currently Applicable Guidelines for the Validation of Analytical Methods 45
3.6.2 Laboratory Proficiency 46
3.6.3 Establishment of Reference Ranges for Candidate Biomarkers 46
3.7 Clinical Qualification of Candidate Biomarkers 47
3.7.1 Methodological Approaches 47
3.7.2 Study Size for Biomarker Performance Characterization 48
3.7.3 Sample Quality and Biobanking 50
3.7.3.1 Sample Collection 50
3.7.3.2 Storage of Sample 51
3.7.3.3 Clinical Data (Sample Annotation) 52
3.7.3.4 Ethical Considerations 53
3.8 Biomarker Qualification in the ‘omics Era 53
3.9 An Example of a Biomarker Provider 54
3.10 Conclusion 55
References 55

4 Biomarker Discovery and Medical Diagnostic Imaging 59
Andreas P. Sakka and James R. Whiteside

4.1 Introduction 59
4.1.1 Imaging Modalities 59
4.1.1.1 Positron Emission Tomography (PET) 59
4.1.1.2 Single Photon Emission Computed Tomography (SPECT) 60
4.1.1.3 Computed Tomography (CT) 60
4.1.1.4 Magnetic Resonance Imaging (MRI) 60
4.1.1.5 Ultrasound (US) 61
4.2 Factors to Consider in Biomarker Selection for Imaging 61
4.3 Defining the Insertion Point of the Assay and Its Business Case 62
4.4 Practical In Vitro Methods Used to Identify Biomarkers 63
4.5 Preclinical Models 64
4.5.1 Model Species 64
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5.2</td>
<td>Inducing Human Disease and Relevant Biomarker Expression</td>
<td>64</td>
</tr>
<tr>
<td>4.5.3</td>
<td>Genetic Manipulation</td>
<td>65</td>
</tr>
<tr>
<td>4.5.4</td>
<td>Pharmacological/Chemical Induction</td>
<td>65</td>
</tr>
<tr>
<td>4.5.5</td>
<td>Xenografts: Grafting Foreign Cells or Tissues</td>
<td>66</td>
</tr>
<tr>
<td>4.5.6</td>
<td>Physical Induction</td>
<td>66</td>
</tr>
<tr>
<td>4.6</td>
<td>Preclinical Analysis Techniques</td>
<td>67</td>
</tr>
<tr>
<td>4.7</td>
<td>Translational Considerations and Restrictions</td>
<td>67</td>
</tr>
<tr>
<td>4.8</td>
<td>Other Uses of Preclinical Models</td>
<td>68</td>
</tr>
<tr>
<td>4.9</td>
<td>Nuclear Imaging Infrastructure</td>
<td>69</td>
</tr>
<tr>
<td>4.10</td>
<td>Image Processing</td>
<td>70</td>
</tr>
<tr>
<td>4.11</td>
<td>Concluding Remarks</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Breath: An Often Overlooked Medium in Biomarker Discovery</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td><em>Jonathan D Beauchamp and Joachim D Pleil</em></td>
<td></td>
</tr>
<tr>
<td>5.1</td>
<td>Introduction</td>
<td>75</td>
</tr>
<tr>
<td>5.1.1</td>
<td>Breath Analysis: Past and Present</td>
<td>76</td>
</tr>
<tr>
<td>5.2</td>
<td>Breath Analysis Studies: Targets, Techniques, and Approaches</td>
<td>77</td>
</tr>
<tr>
<td>5.2.1</td>
<td>Exhaled Breath Gas, Condensate, and Aerosols</td>
<td>79</td>
</tr>
<tr>
<td>5.2.2</td>
<td>Sampling Techniques and Analytical Tools</td>
<td>80</td>
</tr>
<tr>
<td>5.2.3</td>
<td>Discovery Versus Targeted Study Approaches</td>
<td>81</td>
</tr>
<tr>
<td>5.3</td>
<td>Biomarker Confounders</td>
<td>83</td>
</tr>
<tr>
<td>5.3.1</td>
<td>Sampling Impact</td>
<td>83</td>
</tr>
<tr>
<td>5.3.1.1</td>
<td>Online Breath Sampling and Direct Analysis</td>
<td>84</td>
</tr>
<tr>
<td>5.3.1.2</td>
<td>Breath Sampling for Offline Analysis</td>
<td>84</td>
</tr>
<tr>
<td>5.3.2</td>
<td>Contributions from the Exposome</td>
<td>85</td>
</tr>
<tr>
<td>5.4</td>
<td>Biomarkers in Breath</td>
<td>86</td>
</tr>
<tr>
<td>5.4.1</td>
<td>Inorganic Breath Biomarkers</td>
<td>86</td>
</tr>
<tr>
<td>5.4.2</td>
<td>Organic Biomarkers in Breath</td>
<td>87</td>
</tr>
<tr>
<td>5.5</td>
<td>Outlook for Breath Analysis</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>Acknowledgments</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>90</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>HTA in Personalized Medicine Technologies</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td><em>Franz Hessel</em></td>
<td></td>
</tr>
<tr>
<td>6.1</td>
<td>Introduction</td>
<td>95</td>
</tr>
<tr>
<td>6.2</td>
<td>Health Technology Assessment (HTA)</td>
<td>96</td>
</tr>
<tr>
<td>6.3</td>
<td>Validation and Evaluation of Biomarker Tests</td>
<td>99</td>
</tr>
<tr>
<td>6.4</td>
<td>Health Technology Assessment of Personalized Medicine Technologies</td>
<td>100</td>
</tr>
<tr>
<td>6.5</td>
<td>Concluding Remarks</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>105</td>
</tr>
</tbody>
</table>
7  **Bone Remodeling Biomarkers: New Actors on the Old Cardiovascular Stage**  107  
*Cristina Vassalle, Silvia Maffei, and Giorgio Iervasi*

7.1  Introduction  107  
7.2  Cardiovascular Disease and Osteoporosis: Common Risk Factors and Common Pathophysiological Mechanisms  108  
7.3  Biomarkers of Bone Health in CVD  112  
7.3.1  Cathepsin K  112  
7.3.2  Tartrate-Resistant Acid Phosphatase  115  
7.3.3  Sclerostin  115  
7.3.4  Fibroblast Growth Factor 23  116  
7.3.5  Osteopontin  116  
7.3.6  Osteocalcin  117  
7.3.7  Osteoprotegerin  118  
7.3.8  Vitamin D  120  
7.3.9  Other Factors  121  
7.3.10  Genetic Factors  123  
7.4  Conclusion  125  
References  128  

8  **Identification and Validation of Breast Cancer Biomarkers**  147  
*Kori Jackson and Edward Sauter*

8.1  Introduction  147  
8.2  Current Detection and Treatment Modalities  148  
8.2.1  Detection: In Clinical Use  148  
8.2.1.1  Physical Examination  148  
8.2.1.2  Breast Imaging  148  
8.2.2  Detection: Being Evaluated  149  
8.2.2.1  Bodily Fluid Analyses  150  
8.2.3  Treatment: In Clinical Use  150  
8.2.3.1  Surgery and Radiation  150  
8.2.3.2  Systemic Therapy  151  
8.2.4  Treatment: Being Evaluated/Newly Available  153  
8.2.4.1  Biomarkers in Tissue: Single Markers  153  
8.2.4.2  Biomarkers in Tissue: Gene Panels  154  
8.3  Current Biomarker Limitations  154  
8.3.1  Tumor Heterogeneity  154  
8.3.2  Treatment Effect  155  
8.3.3  Primary Versus Recurrent Tumor  155  
8.4  Future Biomarker Discovery Targets  156  
8.4.1  Autoantibodies  156  
8.4.2  Inflammatory Markers  156  
8.4.3  DNA Methylation  157  
8.4.4  Benign Breast Disease  157  
8.4.5  Pregnancy-Associated Breast Cancer  157
9 Evaluation of Proteomic Data: From Profiling to Network Analysis by Way of Biomarker Discovery 163
Dario Di Silvestre, Francesca Brambilla, Sara Motta, and Pierluigi Mauri

9.1 Introduction 163
9.2 Proteomic Methodologies 164
9.3 Shotgun Proteomics 165
9.3.1 Targeted Proteomics 168
9.3.2 Data-Independent Acquisition (DIA) MS 169
9.4 Biomarker Discovery 170
9.4.1 MudPIT Data Processing 172
9.5 Protein–Protein Interaction Network Analysis 174
9.6 Conclusion 176
References 177

10 Biomarkers: From Discovery to Commercialization 183
Sebastian Hoppe and Henry Memczak

10.1 Comparison of Different Platforms 184
10.2 Mass Spectrometry 185
10.3 Enzyme-Linked Immunosorbent Assay 187
10.4 SPR Imaging 188
10.5 Reverse Phase Protein Microarrays 189
10.6 Next-Generation Sequencing (NGS) 190
10.7 Still a Struggle: Achieving Clinical Trial Status 193
10.8 Commercial Biomarker Assays 195
10.9 Quo Vadis, Biomarker Assays? 197
References 199

11 Clinical Validation 207
Mads Almose Røpke

11.1 Introduction 207
11.2 Classification of Biomarkers 208
11.3 Translational Use of Biomarkers 209
11.4 Biomarkers in Clinical Studies 210
## Contents

11.4.1 Healthy Volunteer Studies 210  
11.4.2 Early Patient Studies 211  
11.4.3 Confirmatory Clinical Studies 214  
11.4.4 Enrichment Design 215  
11.4.5 Biomarker-Stratified Design 216  
11.5 Safety Markers in Clinical Development 216  
11.6 Statistical Considerations 218  
11.7 Validation 218  
11.8 Regulatory Considerations for Implementation of Biomarkers in Clinical Studies 221  
11.9 Biorepositories and Ethics 222  
11.10 Conclusion 224  
References 225

12 Genomics and Proteomics for Biomarker Validation 231  
Paula Díez, Rosa Mª Dégano, Nieves Ibarrola, Juan Casado, and Manuel Fuentes  
12.1 Introduction 231  
12.1.1 Biomarker Discovery 233  
12.2 Challenges in Biomarker Discovery/Verification Phases 234  
12.3 Verification of Biomarkers 235  
12.3.1 Protein Binding Assays 235  
12.3.2 Targeted Proteomics 237  
12.3.3 Correlation Between MRM and ELISA 237  
12.3.4 MRM and Biomarker Pipeline 238  
12.4 Role of Biobanking in Biomarkers Validation 238  
12.4.1 Biobanking Challenges Associated with Biomarker Discovery and Validation 239  
12.4.1.1 Preanalytical Variations and Lack of SOPs 239  
12.4.1.2 Biological Diversities 239  
12.4.1.3 Disease Heterogeneity 239  
12.4.1.4 Technical Limitations 240  
12.4.1.5 Validation and Clinical Trials 240  
12.4.1.6 Lack of Stable Biorepository 240  
12.5 Conclusions 240  
References 241  

Index 243