Does Breast Cancer Exist in a State of Chaos?

M. Baum,1 M.A.J. Chaplain,2 A.R.A. Anderson,2 M. Douek1 and J.S. Vaidya1

INTRODUCTION—MODELS OF DISEASE

Throughout the history of medicine mankind has demonstrated extraordinary feats of imagination in elaborating hypothetical models to explain the nature of disease. These models then suggest therapeutic strategies to influence the natural history of disease processes. Until the early 17th Century in Western medicine and to this day in classical Chinese medicine these models were largely metaphysical [1]. However, Ayurveda, the Indian system of medicine, is based on a dynamic balance between three principles: combustion/energy-production/anabolism (Pitta), transport/communication (Vaata), and excretion/catabolism (Kapha). Disease was supposed to be a manifestation of the disturbance of this balance and the doctor’s duty was to maintain and restore this balance [2]. In the last century or so models of disease have progressed from the mechanical to the biological and more recently to the mathematical. The change of allegiance from one model to another has been likened to a Kuhnian revolution or paradigm shift [3]. The two most famous in the history of medicine were the overthrowing of the Galenic doctrine by William Harvey’s description of the circulation of the blood and the replacement of the miasma theory of infection by the bacterial theory of infection which ultimately led to the success of anti-microbial therapy.

MODELS OF BREAST CANCER

Breast cancer, an enigmatic disease with an unpredictable natural history has been a fertile soil for the development of hypothetical models each with their therapeutic consequence. Until the discovery of the cellular nature of cancer, the disease was managed according to Galenic principles, the disease being visualised as an excess of melancholia (black bile) that coagulated within the breast [4]. Treatments aimed at ridding the body of this excess of black bile involved venesection, purgation, cupping, leaching, enemas and bizarre diets (many ‘alternative’ treatments of breast cancer to this day are in fact a form of neo-gallenism).

In the mid 19th Century the humoral theory of breast cancer was overturned by a mechanistic model which described the disease as a phenomenon arising locally within the breast and then spreading centrifugally along lymphatics to be arrested in the first echelon of lymph nodes which acted as a barrier to onward spread by their innate filtering capacity. A second echelon of lymph nodes existed like the casement walls of a medieval town protecting the citadel at its centre. The therapeutic consequence of such a belief was the development of the Halsted radical mastectomy, almost exactly 100 years ago [5].

THE CONTEMPORARY MODEL OF BREAST CANCER

Largely due to the seminal work of Professor Bernard Fisher in the late 1960s, the mechanistic model of the disease was overturned to be replaced by a biological model which looked upon the outcome of the disease as pre-determined by the extent of micrometastases disseminated via the microvasculature of the tumour very early on in its natural history, instead of looking at breast cancer as a chronological event i.e. ‘early’ and ‘late’ we have been taught to look upon breast cancer as a biological challenge with disease being either favourable or unfavourable [6]. The important therapeutic consequence of this belief system has been the development of adjuvant systemic therapy using cytotoxic drugs or endocrine manipulation. This approach has undoubtedly demonstrated the first major impact on breast cancer mortality since the disease was recognised. Unfortunately, the treatments have not lived up to their promise and, although in relative terms, mortality can be reduced by 25%, in absolute terms this translates into a gain of between 5 and 10% over 10–15 years [7, 8]. The pace has now slowed down and fine-tuning of the therapeutic regimens may only offer us incremental points of progress. At the same time, frustrated by this lack of progress, many medical oncologists have been advocating the ‘reductio ad absurdo’ of high-dose chemotherapy and stem cell rescue. Hopes of any important improvement in results with this approach have been dashed with the recent publication of two randomised-controlled trials [9, 10].

FLAWS IN THE CONTEMPORARY MODEL

The triumphalism of the last 20 years of endeavour has now been muted as we begin to recognise the limitations of
adjuvant systemic therapy but along the way we seem to have forgotten one salient feature of the contemporary paradigm. Hidden within the biological model is a linear mathematical model. It has been assumed that the putative micrometastases, that ultimately express themselves as clinical disease, are autonomous and growing according to the mathematical formulae of deterministic or Gompertzian kinetics [11]. Furthermore a second order hypothesis has suggested that in order to achieve maximum cell kill, cytotoxic drugs have to be given at a maximum tolerated dose in cycles to achieve log cell kill until the residual tumour burden can be `mopped up' by the patient's natural host resistance mechanisms [12]. It is now long forgotten that this mathematical model was based on the behaviour of animal experimental systems like the hamster lymphoma described by Skipper and colleagues in the 1960s [13]. What this model fails to explain are the various inconsistencies listed in Table 1. In particular we wish to draw attention to the extraordinary phenomenon of the biphasic nature of hazard rates for relapse and death. Instead of these hazard rates being constant with time, they demonstrate twin peaks, the first at approximately 3 years and a second flatter peak between approximately 7 and 9 years [14,15]. The other extraordinary and chilling fact is that, although the disease-free interval of women who have been operated upon for breast cancer may be extremely variable, ranging from a few weeks to 30 years, once metastases become symptomatic, death is inevitable usually within 2–3 years. Randomised trials which have tested the benefit of regular screening for metastatic disease after treatment of primary breast cancer have failed to show any benefit in terms of mortality [16,17] suggesting that even asymptomatic metastatic disease is not curable at present. This extraordinary phenomenon of dormancy and awakening of metastatic disease has challenged researchers for a long time. Recently, there have been suggestions that the most promising model to explain the dormancy of metastatic disease is the one based on the process of angiogenesis [18].

In this paper, we wish to describe an alternative biological model of metastases in breast cancer that suggests that these are complex organisms existing in a state of dynamic equilibrium close to a chaotic boundary. Furthermore, the mathematics to describe the natural history of these organisms invokes non-linear dynamics or the chaos theory. Central to the understanding of this model has been the pioneering work of Judah Folkman on tumour angiogenesis. [19, 20].

### ANGIOGENESIS AND CANCER

Solid tumours cannot grow beyond $10^6$ cells or approximately 1–2 mm in diameter in the absence of a blood supply [21]. The initial pre-vascular phase of growth is followed by a vascular phase in which tumour-induced angiogenesis is the rate limiting step for further growth and provides malignant cells direct access to the circulation [15,22]. The poor prognostic indication of extensive angiogenesis quantified by microvessel density in histological sections is well recognised in a wide variety of cancers including breast cancer [23,24]. The high vascularity of breast tumours may be imaged by contrast-enhanced magnetic resonance imaging (MRI) since enhancement relies on tumour vascularity and vascular permeability, as demonstrated by histopathologic correlational studies [25] (Figure 1).

The relationship between angiogenesis and tumour cells has been summarised by Folkman's endothelial cell, tumour cell compartment theory. In the tumour cell compartment, cells may stimulate endothelial cell proliferation by the production of tumour angiogenic factors such as beta FGF and VEGF [26]. Alternatively, endothelial cells may themselves stimulate the growth of tumour cells by producing factors such as platelet derived growth factor (PDGF), heparin like growth factor and interleukin 6 (IL6) [27]. It has also been proposed that the primary tumour secretes anti-angiogenic factors and, therefore, removal of the primary tumour might trigger the outgrowth of occult foci into clinically apparent secondary disease [28]. In addition to the importance of the micro-vasculature, we have also to visualise these microscopic foci as existing in a 'soup' of cytokines and endocrine polypeptides and steroids, with cells interacting with each other and with the surrounding stroma. With competing signals directing the cancer cells towards proliferation or apoptosis [18]. It is then possible to visualise sub-clinical metastasis as a complex structure with its future determined by the balance of angiogenic and anti-angiogenic factors as well as factors that stimulate or inhibit epithelial proliferation and stimulate or inhibit apoptosis. Such complexity cannot be modelled by linear dynamics, or even a full understanding of the complete catalogue of genetic mutations at the cellular level, because the critical events of multiple cell to cell interaction requires thorough understanding of epigenetic phenomena. A model

#### Table 1. Inconsistencies and paradoxes in the conventional mechanistic model of breast cancer

<table>
<thead>
<tr>
<th>Breast cancer characteristics</th>
<th>Not linearly related to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour size</td>
<td>Number of lymph nodes involved, i.e.</td>
</tr>
<tr>
<td>Incidence of metastasis at presentation, i.e.</td>
<td></td>
</tr>
<tr>
<td>Hazards of recurrence</td>
<td>Time from diagnosis, i.e.</td>
</tr>
<tr>
<td>Timing of surgery in relation to menstrual period</td>
<td>Survival, i.e.</td>
</tr>
<tr>
<td>Site of metastasis</td>
<td>Vascular or lymphatic access from the primary tumour, i.e.</td>
</tr>
</tbody>
</table>

One can have a large tumour with no lymph nodes involved and an occult primary with axillary lymph nodes involved. Whether the tumour is T1, T2 or even early T3, the incidence of metastases at presentation does not go beyond 5%, but within 2–3 years of diagnosis/therapy, more of the larger tumours present with metastases. The hazards of recurrence and death have a sharp peak at 3 years; the stage at diagnosis only influences the amplitude of the peak but not the timing. The finding that women operated in the follicular phase of their menstrual cycle have a worse prognosis than those operated on in the luteal phase. Tumours can metastasise to either bone or liver bypassing the intervening lung.
somewhat similar to ours has been proposed by Demicheli and colleagues in 1997 [29] and this paper is the first attempt to apply new mathematics of complexity to make predictions about the factors influencing angiogenesis that might one day provide a therapeutic window.

A NEW MATHEMATICAL MODEL FOR BREAST CANCER MICROMETASTASES

Over the last few years several mathematical models have been developed to describe some of the important features of tumour induced angiogenesis. In particular Anderson and Chaplain have described a model using only three important variables involved in tumour angiogenesis [30]. We have called these endothelial cells (EC), tumour angiogenic factors (TAF), and matrix angiogenic factors (MAF). The relatively simple formulae using non-linear dynamics are shown in the appendix and like other ‘chaotic systems’ produce beautiful fractal-like images which can be shown to be exquisitely sensitive to initial conditions (e.g. different concentrations or different gradients of the three biological variables). The 3-dimensional (3-D) mathematical simulation of vascular growth towards a tumour (Figure 2), shows a striking similarity with the vasculature of a breast tumour as visualised using CT (computerised tomography) specimen angiography and 3-D reconstruction (Figure 3). Further illustrations including animations of different simulations can be seen on the internet (http://www.mcs.dundee.ac.uk:8080/~sanderso/ejc99). This type of approach may enable a more accurate means of monitoring response to treatment. Integration of data derived from various imaging modalities (Figure 3) with histopathological tumour assessment in such a non-linear model, might better approximate the natural history of cancer.

HOW DOES THE NEW MODEL EXPLAIN THE INCONSISTENCIES OF THE OLD MODEL?

To explain the late appearance of metastatic disease over a latent period of more than 10 years, we no longer have to invoke a dormant cell theory. It has to be remembered that the cancer cells are not foreign organisms but closer-to-self than non-self [31], and there is no surprise that these complex organisms can exist in a state of dynamic equilibrium until some chance, late event perturbs the status quo. In addition, we can perhaps explain the phenomenon where the breast cancer presents with axillary metastases and the primary is undetectable. The original cancer might have outgrown its blood supply but not before it had a chance to

Figure 1. T1 weighted pre- (a) and post- (b) contrast-enhanced breast MRI: a breast tumour (large arrow) with nipple involvement (small arrow) were clearly visible after contrast enhancement (b). Digital subtraction of image (a) from image (b) and subsequent colour coding (c), demonstrated a very good correlation between areas of high enhancement intensity (red) and areas rich in blood vessels (brown staining) as seen with factor VIII-immunohistochem-

Figure 2. Computer simulation of angiogenesis: computer simulation of the evolution of a capillary network in response to a layer of tumour cells (or alternatively, a large solid circular tumour) over a 15 day period. The simulation shows the migration, branching and anastomosis of the capillary sprouts as they make their way from the parent vessel through the breast tissue.

Figure 3. Three-dimensional CT-reconstruction of breast cancer vascularity. The lateral thoracic artery in a fresh mastectomy specimen was identified and cannulated. A radio-opaque mixture containing barium sulphate was injected into the specimen and a CT scan performed. This 3-dimensional reconstruction of the resulting 106 slices (a) shows the tumour (large orange mass) surrounded by an extensive vascular network (yellow); the small orange mass is the nipple, shown for orientation. The growth pattern of vessels from the lateral thoracic artery (left to right) agree with mathematical predictions of vascular growth, as seen when the tumour and nipple are removed from the image (b).
disseminate cells along the lymphatics, and in the lymph nodes the conditions might exist for the successful establishment of a microvasculature.

The twin peaks can be explained as follows. The removal of the primary tumour disinhbits angiogenesis and encourages epithelial proliferation with the surgical trauma encouraging the secretion of insulin-like growth factor, so in other words the act of surgery kick-starts metastasis. This phenomenon is seen at its most dramatic in women developing breast cancer under the age of 35 years [32]. It could even be argued that if by chance the operation was performed during the follicular phase at a point of maximum epithelial stimulus from oestradiol and minimum apoptotic stimulus through low progesterone levels, then the prognosis might be even worse [33, 34]. Furthermore, it has been demonstrated that tumour expression of genes that govern proliferation and metastatic potential, change during the two phases of menstrual cycle [35]. In other words the system is exquisitely sensitive to initial conditions. The second lower peak could be explained as a stochastic summation of adverse events in a woman’s life such as inter-current infections, second operations or even bereavement.

THERAPEUTIC CONSEQUENCES OF THE NEW MODEL

The specific role of the mathematical model in all of this is to provide objective and quantitative data. The computer simulation that is presented in Figure 2 was realised through applying quantitative estimates of experimentally measurable parameter values such as cell random motility, cell migration rates in response to TAF and MAP gradients etc. The vascular network that appears is, therefore, not only morphologically similar in a qualitative manner (i.e. it looks like a real capillary network) but also, and more importantly, it is morphologically similar in a quantitative manner, i.e. it has the same growth rate as a real network and reaches the tumour in the same time as a real network. Furthermore, the model provides additional data regarding capillary branching patterns, vessel length, numbers of new vessels created, the area and volume of the vessels, etc. Further refinements of the mathematical model include details such as the rate of flow of drugs through its network, interactions with tumour cells and the rate of supply of drugs to the tumour cells. Therefore, it would be possible to incorporate the generally available clinicopathological and radiological data specific to a particular patient into this model which would then provide simulations and predictions that are not directly derivable by standard linear mathematics. Simulating treatments in the model would help to choose the best guess of several possible new treatments.

For example, the therapeutic intervention would be antiangiogenic and the timing of the intervention would be preparative so that at the time of surgery the system was primed to protect against the sudden flooding with angiogenic signals. It might indeed be the case that some of the success attributed to adjuvant tamoxifen is as a result of its anti-angiogenic potential rather than its anti-oestrogenic function [36]. Assuming we can then protect the subject from the first peak of metastatic outgrowth, then we will have to monitor her with extreme vigilance. By the time the metastases are clinically apparent it is perhaps too late, so monitoring the patient with tumour markers and re-introducing an anti-angiogenic strategy at the first rise in tumour markers might prove successful. Better still using PCR techniques to detect variations in the anti-angiogenic milieu may provide an even greater lead time than the use of conventional tumour markers such as CEA and CA15. In the meantime, we can continue to add additional layers of complexity to the simulations of our mathematical model to help develop alternative strategies for biological interventions to maintain the status quo.

Such modeling could potentially precede clinical trials, and accelerate progress of cancer therapeutics. Unlike the hammer lymphoma models of the past, the new model feeds on complexity and gets closer and closer to simulating nature in all its awesome beauty.

In order to achieve the vascularisation of the tumour, the EC have to make their way through the stroma and extracellular matrix which consists of various components including interstitial tissue, collagen fibre, fibronectin and laminin. Various MAfs (e.g. fibronectin, laminin) are known to enhance EC adhesion to collagen and are also produced by EC themselves [37]. EC are known to migrate in response to differential adhesive gradients of MAF created in the surrounding tissue. This type of motion is known as haptotaxis. The EC also detect and respond to changes in the TAF concentration via cell-surface receptors and changes in concentration gradients in the direction of increasing concentration. This type of motion is known as chemotaxis. In addition to these two types of directed motion, we also assume that there is some random motion of the EC.

The mathematical model which we derive considers the development of a vascular bed in response to a solid tumour in the breast (e.g. carcinoma). We consider the domain to be a small cube of breast tissue, the length of each side being 2 mm. Therefore, every point in the tissue has an \((x, y, z)\) Cartesian co-ordinate triple giving its location in the breast tissue space. The concentration of TAF at any point in the breast tissue can thus be described as a function of its location in space and in time and we denote this symbolically by \(c(x, y, z, t)\).

In a similar manner we write the endothelial cell density within the breast tissue as \(n(x, y, z, t)\) and the concentration of MAF within the breast tissue as \(m(x, y, z, t)\). This mathematical description takes account of the fact that these concentrations and cell densities are dynamic, i.e. varying in space and changing with time. We can calculate the rates of change of these variables by using partial differential equations. The mathematical model, therefore, consists of three coupled non-linear partial differential equations describing the evolution in space and time of EC density (random motion, chemotaxis and haptotaxis), TAF concentration (uptake by EC) and MAF concentration (production and uptake by EC) and is given by:

\[
\frac{\partial n}{\partial t} = D \nabla^2 n - \chi \nabla \cdot (n \nabla c) - \rho \nabla \cdot (n \nabla m),
\]

\[
\frac{\partial m}{\partial t} = D \nabla^2 m + \beta n - \rho \nabla \cdot (m \nabla m),
\]

\[
\frac{\partial c}{\partial t} = \beta n - \rho \nabla \cdot (c \nabla m),
\]

where \(D\) is the random motility coefficient, \(\chi\) is the chemotactic response parameter and \(\rho\) is the haptotactic response parameter of the EC, respectively. \(\alpha\) is a measure of the TAF uptake rate by EC, \(\beta\) and \(\gamma\) are measures of the MAF production and uptake rate by EC, respectively. Taking each equation in turn, the terms and equation symbols have the following meanings:

**Equation 1:** The first term in the equation represents the rate of change of EC density with respect to time; the second term in the equation represents the random motility of the EC, i.e. movement of EC in no particular direction; the third term represents the directed migration of EC in response to gradients of TAF, i.e. chemotaxis, the strength of this response measured by the parameter \(\chi\); the final term in the equation represents the directed migration of EC to gradients of MAF within the matrix, i.e. haptotaxis, the strength of this response measured by the parameter \(\beta\).

**Equation 2:** The first term of this equation represents the rate of change of TAF concentration with respect to time; the second term represents the amount of TAF that is being uptaken by the EC, with the rate of uptake being measured by the parameter \(\alpha\).

**Equation 3:** The first term represents the rate of change of MAF concentration with respect to time; the second term represents the amount of MAF produced (synthesised) by the EC, the rate of synthesis being measured by the parameter \(\beta\); the final term represents the amount of MAF being uptaken by the EC, with the rate of uptake being measured by the parameter \(\gamma\).

Parameter estimates were available from experimental observations for the random motility coefficient \(\alpha\) of the EC \((10^{-8}\text{ cm}^2\text{ s}^{-1})\), and the chemotaxis coefficient \(\chi\) \((2600\text{ cm}^2\text{ s}^{-1}\text{ M}^{-1})\) [38, 39].
We solve the above system of mathematical equations using a novel adaptation of a standard numerical analysis technique used in obtaining the numerical solution of partial differential equations—the so-called finite difference method. In essence we firstly discretise the above system of partial differential equations to obtain a new system of discrete equations, and then add an element of stochasticity (randomness). This is accomplished by solving the system of discrete equations via computer simulation on a spatially discrete cube at regular intervals of time. In effect, this means that the $2\text{mm} \times 2\text{mm} \times 2\text{mm}$ breast tissue domain is divided into a meshwork of smaller cubes with sides of 10 microns. At this small length scale the system of partial differential equations is used to generate a set of probabilities for a single cell to move from one location in the meshwork to an adjacent location or to remain stationary. These probabilities reflect the relative influence of the local TAF and MAF concentrations on the endothelial cells. For example, if there exists a local concentration gradient around the cell from left to right (i.e. lower concentration to the left, higher concentration to the right) then the cell will be more likely to move to the right at the next time step. Given the local influences of the TAF and MAF concentrations, a cell then moves up, down, right or left, or remains stationary. This process of generating the cell motion is repeated at each time step of the simulation. We assume that the cell we are tracking is located at the tip of a sprout and hence by following this cell as it moves in response to its surroundings, we in effect simulate the growth of the whole sprout since the remaining cells simply follow the path of the leading EC. In addition to the rules for cell movement we also incorporate rules for sprout branching, anastomosis and EC proliferation. A detailed description of the mathematical techniques and processes used can be found in [30, 38] and at the web-site: http://www.mcs.dundee.ac.uk:8080/~sanderso/angiocont.htm.

Does Cancer Exist in a State of Chaos?