Fanconi’s anaemia and related bone marrow failure syndromes

Inderjeet Dokal

Academic Unit of Paediatrics, Institute of Cell and Molecular Science, Barts and The London, Queen Mary’s School of Medicine and Dentistry, London E1 2AT, UK

The inherited bone marrow (BM) failure syndromes are a heterogeneous group of disorders characterized by BM failure, usually in association with one or more somatic abnormalities. The BM failure may present at birth or at a variable time thereafter including in adulthood in some cases. Over the last decade, there have been significant advances in the genetics of these syndromes particularly Fanconi’s anaemia (FA) and dyskeratosis congenita (DC). These advances are beginning to provide a better understanding of normal haemopoiesis and of the pathophysiology of some cases of idiopathic aplastic anaemia (AA). They have also provided important insights into some aspects of DNA repair (FA/BRCA pathway) and telomere maintenance (DC-related genes), two pathways critical in the maintenance of genomic stability. These advances are already facilitating better diagnosis of patients with these disorders. It is hoped that they will also form the basis for developing new treatments.

Keywords: bone marrow failure; DNA repair; dyskeratosis congenita; Fanconi’s anaemia; telomerase

Introduction

Bone marrow (BM) failure syndromes are characterized by the inability of the BM to produce an adequate number of circulating blood cells. They are associated with significant mortality due to bleeding or infection. The vast majority (~70%) of these cases are classified as idiopathic as their primary aetiology remains unexplained (Table 1). In a subset (~15% of cases), a drug or infection can be identified that precipitates the BM failure/aplastic anaemia (AA), although it is not clear why only some individuals are susceptible. In ~10–20% of patients (the focus of this review), the disease is constitutional/inherited, where the disease is familial and/or presents with one or more other somatic abnormalities [1] (Table 2). The features of some of the classical syndromes are summarized in Table 3. The precise incidence/prevalence of these remains...
**Table 1** The bone marrow failure syndromes—‘Conventional’ classification

<table>
<thead>
<tr>
<th>Classification</th>
<th>Range (%)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic</td>
<td>-70%</td>
<td>Vast majority</td>
</tr>
<tr>
<td>Inherited</td>
<td>-10-20%</td>
<td>Dyskeratosis congenita</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fanconi’s anaemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shwachman–Diamond syndrome</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others</td>
</tr>
<tr>
<td>Secondary</td>
<td>-10-15%</td>
<td>Radiation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Predictable*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total body irradiation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Predictable*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Busulphan</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Idiosyncratic†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-steroidals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Idiosyncratic†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Viruses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hepatitis viruses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Immunity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>As part of SLE</td>
</tr>
</tbody>
</table>

SLE, systemic lupus erythematosus.

*These agents produce bone marrow (BM) failure in all patients if used at a high dose.

†The BM failure only develops in some individuals following exposure to the agent, suggesting that there may be a genetic predisposition to the development of BM failure.

**Table 2** The inherited bone marrow failure syndromes

- Usually associated with trilineage haemopoietic defect
  - Fanconi’s anaemia (FA)
  - Dyskeratosis congenita (DC)
  - Shwachman–Diamond syndrome (SDS)
  - Pearson syndrome (PS)
  - Familial aplastic anaemia (autosomal and X-linked forms)

- Usually associated with single lineage haemopoietic defect
  - Anaemia
    - Diamond–Blackfan anaemia (DBA)
  - Neutropenia
    - Severe congenital neutropenia (SCN) including Kostmann syndrome
  - Thrombocytopenia
    - Congenital amegakaryocytic thrombocytopenia (CAMT)
    - Amegakaryocytic thrombocytopenia with absent radii (TAR)

**Table 3** Characteristics of the inherited bone marrow failure syndromes

<table>
<thead>
<tr>
<th></th>
<th>FA</th>
<th>DC</th>
<th>SDS</th>
<th>DBA</th>
<th>TAR</th>
<th>SCN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inheritance pattern</td>
<td>AR, XLR</td>
<td>XLR, AR, AD</td>
<td>AR</td>
<td>AD</td>
<td>AR</td>
<td>AD</td>
</tr>
<tr>
<td>Somatic abnormalities</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Rare</td>
</tr>
<tr>
<td>Bone marrow failure</td>
<td>AA (&gt;90%)</td>
<td>AA (&lt;80%)</td>
<td>AA (20%)</td>
<td>RCA</td>
<td>Megas</td>
<td>Neut</td>
</tr>
<tr>
<td>Short telomeres</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Cancer</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>?</td>
<td>No</td>
</tr>
<tr>
<td>Chromosome instability</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Genes identified</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>No</td>
<td>1</td>
</tr>
</tbody>
</table>

AA, aplastic anaemia; AD, autosomal dominant; AR, autosomal recessive; DBA, Diamond–Blackfan anaemia; DC, dyskeratosis congenita; FA, Fanconi’s anaemia; Megas, low megakaryocytes; Neut, neutropenia; RCA, red cell aplasia; SCN, severe congenital neutropenia; SDS, Shwachman–Diamond syndrome; TAR, thrombocytopenia with absent radii; XLR, X-linked recessive.
unclear. The BM failure (may involve all or a single lineage) may present at birth or at a variable time thereafter including in adulthood in some cases. Scientifically, they constitute an important group of disorders as recent advances in the genetics of some of these are not only beginning to unravel their pathophysiology but are also providing important insights into normal haemopoiesis, DNA repair and telomere biology.

**Fanconi’s anaemia**

**Clinical features**

Fanconi’s anaemia (FA) is an inherited (usually autosomal recessive but rarely X-linked recessive) disorder in which there is progressive BM failure and an increased predisposition to cancer [1–23]. Affected individuals may also have one or more somatic abnormalities including skin, skeletal, genitourinary, gastrointestinal, cardiac and neurological anomalies. Approximately, a third of FA patients have no somatic abnormalities. Most patients present towards the end of the first decade of life and die as young adults from complications of BM failure or malignancy. However, increasingly some patients are being diagnosed in adulthood.

**Molecular and cell biology**

Cells from FA patients usually show an abnormally high frequency of spontaneous chromosomal breakage and hypersensitivity to the clastogenic effect of DNA cross-linking agents such as diepoxybutane (DEB) and mitomycin-C (MMC). A diagnostic test, based on the increased chromosomal breakage seen in FA cells compared with normal controls after exposure to DEB or MMC (DEB/MMC stress test), is available. Other features of the FA cell phenotype include abnormal cell cycle kinetics (prolonged G2 phase), hypersensitivity to oxygen, increased apoptosis and accelerated telomere shortening.

There is considerable genetic heterogeneity in FA with 12 subtypes/complementation groups (A, B, C, D1, D2, E, F, G, I, J, L and M) currently recognized [4]. The genes for 11 (FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCJ, FANCL and FANCM) of these subtypes have been identified. It has also been observed recently that although FA is usually an autosomal recessive disorder in a small subset of patients, it is X-linked (FANCB subgroup). The approximate prevalence of the different FA subgroups is summarized in Table 4.
Studies over the last 10 years have demonstrated that the FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL and FANCM proteins interact with each other and form a nuclear complex called the ‘FA core complex’ (Fig. 1). The identification of the FANCD2 gene provided an important link between the FA proteins and DNA repair [5]. The FA core complex containing the FA proteins (A/B/C/E/F/G/L/M) is required for the activation of the FANCD2 protein to a monoubiquitinated isoform (FANCD2-Ub). In normal (non-FA) cells, FANCD2 is monoubiquitinated in response to DNA damage and is targeted to chromatin containing the DNA damage (e.g. DNA cross-link). FANCD2-Ub then interacts with DNA repair proteins (including BRCA2, BRCA1 and RAD51) leading to repair of the cross-link. In cells from A, B, C, E, F, G, L or M patients, FANCD2 ubiquitination is not observed. Recently, it has been shown that cell lines derived from FA-D1 patients have biallelic mutations in BRCA2 [6]. This observation has linked the FA proteins (FANCA, FANCB, FANCC, FANCE, FANCD2, FANCE, FANCF, FANCG, FANCL and FANCM) with BRCA1 and BRCA2 in a common pathway. The BRCA2 protein is important in the repair of DNA damage by homologous recombination (HR). Cells lacking BRCA2 inaccurately repair damaged DNA and are hypersensitive to DNA cross-linking agents. These recent findings therefore suggest that BRCA2 and other FA proteins cooperate in a common DNA-damage response pathway, the ‘FA/BRCA pathway’. It has also been observed that FANCJ is BRIP1, which interacts with BRCA1. These new findings further highlight the connection between the FA and BRCA proteins and DNA repair [7]. Disruption of this FA/BRCA pathway results in the cellular and clinical phenotype common to all FA subtypes. There is recent

---

**Table 4** Fanconi’s anaemia (FA) complementation groups/genetic subtypes

<table>
<thead>
<tr>
<th>Complementation group (gene)</th>
<th>Approximate % of FA patients</th>
<th>Chromosome location</th>
<th>Protein (amino acids)</th>
<th>Exons</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (FANCA)</td>
<td>66</td>
<td>16q24.3</td>
<td>1455</td>
<td>43</td>
</tr>
<tr>
<td>B (FANCB)</td>
<td>&lt;1</td>
<td>Xq22.2</td>
<td>859</td>
<td>10</td>
</tr>
<tr>
<td>C (FANCC)</td>
<td>12</td>
<td>9q22.3</td>
<td>558</td>
<td>14</td>
</tr>
<tr>
<td>D1 (FANCD1*)</td>
<td>&lt;1</td>
<td>13q12.3</td>
<td>3418</td>
<td>27</td>
</tr>
<tr>
<td>D2 (FANCD2)</td>
<td>&lt;1</td>
<td>3p25.3</td>
<td>1451</td>
<td>44</td>
</tr>
<tr>
<td>E (FANCE)</td>
<td>4</td>
<td>6p21.1</td>
<td>536</td>
<td>10</td>
</tr>
<tr>
<td>F (FANCF)</td>
<td>4</td>
<td>11p15</td>
<td>374</td>
<td>1</td>
</tr>
<tr>
<td>G (FANCG)</td>
<td>12</td>
<td>9p13</td>
<td>622</td>
<td>14</td>
</tr>
<tr>
<td>I (FANCI)</td>
<td>&lt;1</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>J (FANCJ/BRIP1)</td>
<td>&lt;5</td>
<td>17q23.1</td>
<td>1249</td>
<td>20</td>
</tr>
<tr>
<td>L (FANCL)</td>
<td>&lt;1</td>
<td>2p16.1</td>
<td>375</td>
<td>14</td>
</tr>
<tr>
<td>M (FANCM)</td>
<td>&lt;1</td>
<td>14q21.3</td>
<td>2048</td>
<td>23</td>
</tr>
</tbody>
</table>

*FANCD1 is BRCA2.
†FANCB is on the X-chromosome.
data to suggest that the FA/BRCA pathway is activated in response to DNA damage (replication fork arrest) by ataxia–telangiectasia and RAD3-related protein (ATR) (Fig. 1). The pathway is inactivated by the deubiquitinating enzyme, USP1. ATR is mutated in a subfamily of Seckel’s syndrome, a disease exhibiting some clinical similarity to FA. ATR and ataxia–telangiectasia-mutated (ATM) proteins are known to phosphorylate FANCD2. ATR–Seckel cells also exhibit defects in FANCD2 monoubiquitination. Furthermore, Nijmegen breakage syndrome (NBS) cells (mutated in NBS) show defects in FANCD2 monoubiquitination. This as well as clinical overlap between FA, NBS and Seckel patients suggests that there is also overlap in the biological defects observed in cells from these patients. Thus, although many of the steps involved in the FA/BRCA pathway have now been elucidated, gaps still remain in our understanding of the precise physical interactions between the different molecules involved in this pathway(s).
Treatment

The main cause of premature mortality in FA patients is the development of BM failure. Anabolic steroids such as oxymetholone can produce useful trilineage (erythroid, myeloid and megakaryocytic lineages) haematological responses in 50–70% of patients, but the majority will become refractory after a variable period. Nevertheless, oxymetholone is a good holding treatment until definitive treatment can be planned using haemopoietic stem cell transplantation (SCT). From the in vitro and in vivo studies it has become clear that cells from FA patients are hypersensitive to agents such as cyclophosphamide and irradiation compared with non-FA patients. Therefore, SCT-conditioning regimens have been modified by reducing the dose of cyclophosphamide and radiation. Using low-dose cyclophosphamide (20 mg/kg) and 4.5–6 Gy of total body irradiation, the actuarial survival for patients transplanted using human leucocyte antigen (HLA)-identical sibling donors is around 70% at 2 years [8]. The results using unrelated donors are less good with 2-year survival between 20 and 40% [9]. In order to improve these results, conditioning protocols using Fludarabine are being investigated. Additionally, long-term follow-up of patients who have survived following SCT shows a much higher incidence of malignancies, particularly of the head and neck usually 8–10 years after the transplant. This in part relates to the inherent predisposition of FA patients to malignancy and in part to factors such as the use of radiotherapy in the conditioning. Results using Fludarabine-based protocols, which avoid radiotherapy, seem to be encouraging for both sibling and unrelated stem cell transplants [10, 11]. Fludarabine, a fluorinated analogue of adenosine arabinoside, has significant immunosuppressive activity (reduces host lymphocyte counts) and is able to facilitate engraftment of donor cells without the need for myeloablative conditioning.

The identification of the FA genes combined with the in vitro gene transfer data, which show that FA haemopoietic stem cells rescued by gene therapy should have a selective growth advantage within the hypoplastic BM environment, has resulted in a pilot study of retroviral-mediated gene therapy for FA-C patients. To date, this study has recruited four FA-C patients, and the preliminary results are encouraging, with no serious side effects reported [12]. However, gene therapy for FA is not yet a reality in the clinic.

Additional evidence, which strengthens the case for future trials of gene therapy, comes from reports of FA mosaic patients. In such cases, the DEB/MMC test may be negative or only demonstrate chromosomal hypersensitivity in a subgroup of cells. Somatic mosaicism is due to reversion of a pathogenic allele to ‘wild’ type in a single haemopoietic (somatic) cell. The mechanism of how this occurs can vary [13], but in
each case it generates one ‘functionally normal’ FA allele, and the result-
ing cell effectively becomes a ‘heterozygous cell’ which would be ex-
pected to have a growth/survival advantage in the background of FA 
cells. These mosaic patients have been in haematological remission, sug-
gesting that a single pluripotent stem cell may be sufficient to restore 
adequate haemopoiesis. FA patients with somatic mosaicism can thus be 
regarded as having undergone ‘natural haemopoietic gene therapy’.

**Dyskeratosis congenita**

*Clinical features*

Classical dyskeratosis congenita (DC) is an inherited disease character-
ized by the triad of abnormal skin pigmentation, nail dystrophy and 
mucosal leucoplakia [14]. Variety of non-mucocutaneous (dental, gas-
trointestinal, genitourinary, neurological, ophthalmic, pulmonary and 
skeletal) abnormalities has also been reported. BM failure is the major 
cause of early mortality, with an additional predisposition to mali-
gnancy and fatal pulmonary complications. X-linked recessive, auto-
osomal dominant and autosomal recessive forms of DC are recognized.

Clinical features in DC often appear during childhood. The skin pig-
mentation and nail changes typically appear first, usually by the age of 
10 years. BM failure usually develops below the age of 20 years; 80–90% 
of patients will have developed BM abnormalities by the age of 30 years. 
In some cases, the BM abnormalities may appear before the mucocuta-
neous manifestations. The main causes of death are BM failure/immuno-
deficiency (~60–70%), pulmonary complications (~10–15%) and 
malignancy (~10%).

*Molecular and cell biology*

Lymphocytes from DC patients generally show no significant difference 
in chromosomal breakage compared with those from normal controls, 
with or without the use of bleomycin, DEB, MMC and γ-irradiation. 
Primary DC skin fibroblasts are abnormal, both in morphology and in 
growth rate. They are also predisposed to developing unbalanced chro-
mosomal rearrangements (dicentrics, tricentrics and translocations) in 
the absence of any clastogenic agents. In addition, peripheral blood (PB) 
and BM metaphases from some patients show unbalanced chromosomal 
rearrangements in the absence of any clastogenic agents. These studies 
provide evidence for a defect, which predisposes DC cells to developing 
chromosomal rearrangements. DC, like FA, may thus be regarded as a 
chromosomal instability disorder.
Haemopoietic progenitor studies have shown reduced numbers of all types of progenitors (consistent with a haemopoietic defect at the stem cell level) compared with controls. The degree to which the progenitors are reduced can vary from patient to patient, and they can be reduced even when the PB count is normal. The demonstration of abnormalities of growth and chromosomal rearrangements in fibroblasts suggests that the BM failure is likely to be a consequence of abnormalities in both haemopoietic stem cells and stromal cells.

X-chromosome inactivation patterns (XCIPs) have been studied in PB cells of women from X-linked DC families by investigating a methylation-sensitive restriction-enzyme site in the polymorphic human androgen receptor locus at Xq11.2–Xq12 (HUMARA). All carriers of X-linked DC showed complete skewing in XCIP. The presence of the extremely skewed pattern of X-inactivation in PB cells suggests that cells expressing the defective gene have a growth/survival disadvantage over those expressing the normal allele.

Linkage analysis performed in one large family with only affected males made it possible to map the gene for the X-linked form of DC to Xq28 [15]. The availability of genetic markers and additional X-linked families facilitated positional cloning of the gene (DKC1) that is mutated in X-linked DC [16]. The identification of the DKC1 gene has made available a genetic test that can be used to confirm diagnosis in suspected cases and antenatal diagnosis in X-linked families. It has also led to the demonstration that the Hoyeraal–Hreidarsson (HH) syndrome is also due to mutations in the DKC1 gene [17]. HH is a severe multi-system disorder characterized by severe growth failure, abnormalities of brain development, AA and immunodeficiency.

The DKC1 gene is expressed in all tissues of the body, indicating that it has a vital function in the human cell. This correlates well with the multi-system phenotype of DC. The DKC1 gene and its encoded nucleolar protein, dyskerin, are highly conserved throughout evolution. On the basis of strong similarity to corresponding proteins in other organisms, it is predicted that dyskerin is associated with the hairpin-Hinge-hairpin/ACA (H/ACA) class of small nucleolar RNAs (snoRNAs) and is involved in pseudouridylation (conversion of uracil to pseudouracil) of specific residues of ribosomal RNA (rRNA). This step is essential for ribosome biogenesis and therefore initially suggested that DC arises largely because of a problem with ribosome biogenesis.

Subsequent studies have shown that dyskerin also associates with the RNA component of telomerase (TERC; Fig. 2) which also contains a H/ACA consensus sequence [18]. Telomerase is an enzyme complex that is important in maintaining chromosomal telomere length after cell division. The precise composition of the telomerase complex is unknown, but its two essential components, the RNA component (TERC) and the
catalytic reverse transcriptase (TERT), have been well characterized. In patients with X-linked DC, it was demonstrated that the level of TERC was reduced and that telomere lengths were much shorter than in age-matched normal controls. Subsequently, it was found that telomeres are also shorter in cells from patients with autosomal forms of DC. This therefore suggested that DC might be a disease of telomere maintenance rather than ribosomal biogenesis. Further clarification came from another genetic development: linkage analysis in one large DC family showed that the gene for autosomal dominant DC is on chromosome 3q, in the same area where the gene for TERC had been previously mapped. This led to TERC mutation analysis in this and other DC families and the demonstration that autosomal dominant DC is due to mutations in the TERC gene [19].

Because the DKC1-encoded protein dyskerin and TERC are both key components of the telomerase complex, it now appears that DC arises principally from an abnormality in telomerase activity. The brunt of the

---

**Fig. 2** Schematic representation of the interaction between dyskerin and the other core molecules (GAR1, NHP2, NOP10, TERC and TERT) of the telomerase complex. This is a RNA–protein complex, because TERC is a 451b RNA molecule which is never translated. The other molecules (dyskerin, GAR1, NHP2, NOP10 and TERT) are proteins; their molecular weights are shown. In vitro, telomerase activity can be reconstituted by expressing TERC and TERT alone. Dyskerin, GAR1, NHP2 and NOP10 are believed to be important for the stability of the telomerase complex.
Dyskeratosis congenita (DC) disease falls on tissues that need constant renewal, consistent with a basic deficiency in stem cell activity due to defective telomerase activity. The demonstration of DKC1 and TERC mutations in DC families provides an accurate diagnostic test, including antenatal diagnosis, in a significant subset of cases (Table 5). For DC patients, this now provides the basis for designing new treatments. Scientifically, it provides the first genetic link between a human disease that is characterized by features of premature ageing (premature grey hair/hair loss, BM failure, increased predisposition to malignancy) and short telomeres. Therefore, unraveling the biology of DC has had important implications not only for patients with DC but also for the age-related disorders such as cancer and AA which too are associated with abnormal telomeres.

Recently, TERC mutations have been observed in a subset of patients with AA, myelodysplasia (MDS) and paroxysmal nocturnal haemoglobinuria (PNH) but who lacked classical features of DC [20–22]. Furthermore, AA patients associated with TERC mutations had significantly shorter telomeres than age-matched controls. These data indicate that in a subset of patients with AA, MDS and PNH, the disorder is associated with a genetic defect in the telomere maintenance pathway. It also suggests treatments aimed at restoration of telomere length might be useful in this group of patients. Heterozygous mutations in TERT [23–25] have been recently found in some patients with BM failure and DC. These findings further support the model that DC is principally a disorder of telomere maintenance.

**Treatment**

As in FA, oxymetholone can produce an improvement in haemopoietic function in some patients for a variable time. Transient successful responses to granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage CSF (GM-CSF) and erythropoietin have also been reported. The main treatment for severe BM failure, however, is allogeneic SCT, and there is some experience using both sibling and alternative stem cell donors. Unfortunately, because of early and late fatal

<table>
<thead>
<tr>
<th>Table 5 Dyskeratosis congenita (DC) genetic subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC subtype</td>
</tr>
<tr>
<td>X-linked recessive</td>
</tr>
<tr>
<td>Autosomal dominant</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Uncharacterized*</td>
</tr>
</tbody>
</table>

*These are likely to represent more than one genetic locus and include autosomal recessive (AR) DC.
pulmonary/vascular complications following SCT, the results of allogeneic SCT have been less successful than in FA. The presence of pulmonary disease in a proportion of DC patients explains the high incidence of fatal pulmonary complications in the setting of SCT. It also highlights the need to avoid agents that are associated with pulmonary toxicity (e.g. radiotherapy and busulphan). Because BM failure is the main cause of premature death in DC patients and SCT is currently the only curative option for the BM failure, SCT should continue to be performed on carefully selected patients. The best candidates for SCT are patients with no pre-existing pulmonary disease and who have sibling donors. SCT using Fludarabine-based protocols appears to be giving encouraging preliminary results.

In the future, it will be interesting to determine whether treatments (e.g. by transfer of wild-type TERC or DKC1 into DC haemopoietic cells) aimed at restoration of telomere length will be possible.

**Shwachman–Diamond syndrome**

**Clinical features**

Shwachman et al. and Bodian et al. first reported this disease independently in 1964 [26, 27]. It is now recognized as an autosomal recessive disorder characterized by exocrine pancreatic insufficiency, BM dysfunction and other somatic abnormalities (particularly metaphyseal chondrodysplasia) [28]. Features of pancreatic insufficiency (malabsorption, failure to thrive) are apparent early in infancy. (The pancreatic function can improve in some Shwachman–Diamond syndrome [SDS] patients by 4 years of age.) Other common features include short stature, protuberant abdomen and an ichthyotic skin rash. Metaphyseal dysostosis is seen on radiographs in ∼75% of patients. Hepatomegaly, rib/thoracic cage abnormalities, hypertelorism, syndactyly, cleft palate, dental dysplasia, ptosis and skin pigmentation may also be observed in some patients.

The spectrum of haematological abnormalities includes neutropenia, other cytopenias (∼20% have pancytopenia), MDS and leukaemic transformation (∼25%). The age at which leukaemia develops varies widely from 1–43 years. Acute myeloid leukaemia is the commonest category, and there is a preponderance of cases of leukaemia in males (M : F; ∼9:1). At present, there is no explanation for this sex difference.

**Molecular and cell biology**

The SDS gene (SBDS) within 7q11 was identified in 2003 [29]. The majority (>90%) of SDS patients have been found to have mutations in this gene.
The precise function of the protein encoded by the *SBDS* gene is unknown, but based on the function of its homologues, it is predicted to have an important role in RNA metabolism and/or ribosome biogenesis [30]. Several abnormalities in SDS cells have been observed, including haemopoietic stem and stromal defects, increased rates of apoptosis and short telomeres. It will be important to determine how mutations in the *SBDS* gene lead to all these cell defects, the increased frequency of isochromosome 7q [i(7q)] and the clinical abnormalities characteristic of SDS. More immediately, this advance provides a genetic test facilitating accurate diagnosis.

**Treatment**

The malabsorption in SDS responds to treatment with oral pancreatic enzymes. For those with neutropenia, G-CSF may produce an improvement in the neutrophil count. Some patients with anaemia and/or thrombocytopenia may achieve haematological responses with oxymetholone. As in other types of BM failure, supportive treatment with red cell and platelet transfusions and antibiotics is very important. The main cause of death is infection or bleeding.

Recent analysis of SDS patients has shown that the incidence of MDS and transformation to acute myeloid leukaemia (∼15–25%) is higher than previously reported. The development of leukaemia, often with features of MDS, usually has a poor prognosis. SDS patients with leukaemia treated with conventional courses of chemotherapy usually fail to regenerate normal haemopoiesis. Because this is a constitutional disorder, all somatic cells, including haemopoietic stem cells, are abnormal. In addition, the haemopoietic stem cells may have accumulated secondary abnormalities as suggested by complex karyotypes (especially involving chromosome 7) often observed in the BM from such patients. Therefore, for those who develop leukaemia, usually the only successful treatment is allogeneic SCT [31]. In the future, conditioning regimens that include Fludarabine might be associated with improved outcome. The similarities between SDS and the other inherited BM failure syndromes emphasize that SDS should be regarded as a disorder with high propensity to develop both AA and leukaemic transformation. Because these complications may not develop until adult life, it is important to continue follow-up throughout life.

**Diamond–Blackfan anaemia**

**Clinical features**

Red cell aplasia was first reported in 1936 by Josephs. In 1938, Diamond reported on four children with hypoplastic anaemia, and this has now
come to be recognized as Diamond–Blackfan anaemia (DBA) or congenital pure red cell aplasia. DBA usually presents in early infancy with symptoms of anaemia such as pallor or failure to thrive. The hallmark of classical DBA is a selective decrease in erythroid precursors and normochromic macrocytic anaemia associated with a variable number of somatic abnormalities such as craniofacial, thumb, cardiac and urogenital malformations. Conventional haematological diagnostic criteria for DBA have included (i) normochromic, usually macrocytic, but occasionally normocytic anaemia developing in early childhood; (ii) reticulocytopenia; (iii) normocellular BM with selective deficiency of erythroid precursors (erythroblasts <5%); (iv) normal or slightly decreased leucocyte counts and (v) normal or often increased platelet counts. More recently, an elevated erythrocyte deaminase activity, macrocytosis and an elevated foetal haemoglobin (Hb) have been added to the list of supportive features of DBA.

There is considerable heterogeneity in the associated somatic abnormalities and response to therapy. Informative analysis of 420 cases recruited to the DBA registry of North America (DBAR) has been published recently [32]. The annual incidence of DBA is ~5 per million live births. The median age at presentation was 8 weeks; 93% of patients presented in the first year of life. Seventy-nine per cent of patients were initially responsive to steroids, 17% were non-responsive, and 4% were never treated with steroids. Thirty-one per cent of patients were receiving transfusions at analysis. The actuarial survival rate at older than 40 years was 100% for those in sustained remission, 87% for steroid-maintained patients and 57% for transfusion-dependent patients. Of the 36 deaths reported to the registry, 25 were treatment related: 5 from infections, 5 from complications of iron overload, 1 vascular access-related death and 14 from transplant-related complications.

In the DBAR, 8.8% of families had more than one affected individual. Most of the familial cases displayed autosomal dominant pattern of inheritance. Somatic anomalies, excluding short stature, were found in 47% of patients. Of these, 50% were of the face and head (high arched palate, cleft lip, hypertelorism and flat nasal bridge), 38% were of upper limb and hand (flat thenar eminence, triphalangeal thumb), 39% genitourinary, 30% cardiac. Height was below the third centile for age in ~30%.

MDS and acute myeloid leukaemia (AML) have been reported in a few patients with DBA, suggesting an increased predisposition to haematological malignancies. Non-haematological malignancies (e.g. osteosarcoma) have also been observed. There are also cases that have evolved into AA; neutropenia and thrombocytopenia are relatively common after the first decade. Giri et al. [33] reported on moderate to severe BM hypocellularity in 21/28 (75%) with steroid-refractory DBA; marrow
hypoplasia correlated with the development of neutropenia (9/21; 43%) and/or thrombocytopenia (6/21; 29%). Furthermore, using *in vitro* long-term culture-initiating cell (LTC-IC) assay, they provided evidence for a trilineage haemopoietic defect in patients with refractory DBA. Thus, although classically DBA has been regarded as a pure red cell aplasia, a more global haemopoietic defect is likely to be present, and this may be seen more frequently in the future as patients are surviving longer because of improved medical care.

**Molecular and cell biology**

The classical haematological profile in DBA patients consists of normochromic macrocytic anemia, reticulocytopenia and a normocellular marrow with selective deficiency of red cell precursors. A number of different defects of *in vitro* erythroid progenitor proliferation, differentiation, apoptosis and cytokine responsiveness have been reported but have not clarified the mechanism of *in vivo* erythroid failure. For many years, based on the typical selective deficiency in red cell precursors, many researchers believed that DBA is due to an intrinsic problem with erythroid proliferation/differentiation. On the contrary, the observation of a wide range of somatic abnormalities in a significant proportion of patients and reports of thrombocytopenia, neutropenia and AA together with the recent evidence for a trilineage haemopoietic defect suggest that the primary problem in DBA is not just confined to the erythroid lineage.

The establishment of DBA registries, recent advances in genetics and the identification of a female with a balanced X;19 translocation are facilitating a better understanding of DBA. Recent data from the UK show that ~45% of DBA cases are familial [34]. The first DBA gene *DBA1* (*RPS19*) was identified in 1999 [35]. The *RPS19* gene is located at 19q13.2 and encodes the ribosomal protein RPS19. Analysis of 172 DBA families (190 patients) by the DBA Working Group of the European Society of Paediatric Haematology and Immunology (ESPHI) has demonstrated heterozygosity for mutations affecting the *RPS19* gene in 42 of 172 index patients (24.4%) [36], thus confirming the genetic heterogeneity of DBA. Interestingly, mutations in the *RPS19* gene were also found in some apparently unaffected individuals from DBA families, presenting only with an isolated elevation of erythrocyte adenosine deaminase activity (eADA). The lack of a genotype–phenotype correlation implies that other factors modulate the phenotypic expression of the primary genetic defect in families with *RPS19* mutations.

Furthermore, linkage analysis in DBA families has identified a second DBA locus on chromosome 8p23.–22 to which ~40% of DBA families map. However, a significant proportion of families map to neither the
locus on chromosome 8 nor 19, suggesting that there are likely to be at least three DBA genes.

The \textit{RPS19} gene shows a ubiquitous expression profile and encodes a 145-amino acid protein with a predicted molecular weight of 16 kDa. This protein has significant homologies with proteins from diverse species. Its precise function remains unknown, although it is predicted to have a role in ribosome biogenesis [37].

The demonstration of \textit{RPS19} mutations in \textasciitilde 25\% of DBA families now makes it possible to confirm the diagnosis in a subset of DBA patients. This is useful in counselling families. However, the observed poor genotype–phenotype correlation means predictions regarding prognosis are not easy.

\textbf{Treatment}

The first line treatment for DBA remains corticosteroids. Once a maximal Hb response has been achieved, the dose of prednisolone should then be tapered slowly until the patient is on the lowest dose possible on an alternate day regimen. The dose required to achieve this can vary considerably from patient to patient. For those patients who fail to respond or become refractory to steroids, blood transfusion is the mainstay of treatment. As in thalassaemia major, the main complication from transfusions is iron overload, and iron chelation with desferrioxamine should therefore be commenced as soon as patients have increased iron stores. The promising results with the new oral iron chelator, deferasirox/ICL670, are likely to have a positive impact on the management of this group of DBA patients. Splenectomy may be indicated in the event of an increased transfusion requirement secondary to hypersplenism. For patients who are transfusion dependent and who have a compatible sibling donor, haemopoietic SCT may be appropriate and is potentially curative.

\textbf{Conclusion}

Over the last decade, there have been significant advances in the genetics of the inherited BM failure syndromes, particularly FA and DC. These advances are beginning to provide a better understanding of normal haemopoiesis and of the pathophysiology of idiopathic AA. They have also provided important insights into some aspects of DNA repair (the FA/BRCA pathway) and telomere maintenance (DC-related genes). These advances are already facilitating better diagnosis of patients with these disorders. It is hoped that they will also form the platform for developing new treatments.
Acknowledgements

I thank my current (Richard Beswick, Michael Kirwan, Anna Marrone, Amanda Walne and Tom Vulliamy) and past colleagues (Stuart Knight, Philip Mason and David Stevens) whose contribution has been critical over the years to DC research. I am also grateful to the DC families and all our colleagues (doctors and nurses) for their support in establishing the Dyskeratosis Congenita Registry and to the MRC and Wellcome Trust for financial support.

References