3 IRON OVERLOAD AND CHELATION

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Iron overload occurs when iron intake is increased over a sustained period of time, either as a result of red blood cell transfusions or increased absorption of iron through the gastrointestinal (GI) tract. Both of these occur in thalassaemias, with blood transfusion therapy being the major cause of iron overload in thalassaemia major and increased GI absorption being more important in non-transfusion dependent thalassaemia (NTDT). When thalassaemia major patients receive regular blood transfusion, iron overload is inevitable because the human body lacks a mechanism to excrete excess iron. Iron accumulation is toxic to many tissues, causing heart failure, cirrhosis, liver cancer, growth retardation and multiple endocrine abnormalities.

Chelation therapy aims to balance the rate of iron accumulation from blood transfusion by increasing iron excretion in urine and or faces with chelators. If chelation has been delayed or has been inadequate, it will be necessary to excrete iron at a rate which exceeds this. Because iron is also required for essential physiological purposes, a key challenge of chelation therapy is to balance the benefits of chelation therapy with the unwanted effects of excessive chelation. Careful dose adjustment is necessary to avoid excess chelation as iron levels fall. The second major challenge in chelation therapy is to achieve regular adherence to treatment regimens throughout a lifetime, as even short periods of interruption to treatment can have damaging effects. While the convenience and tolerability of individual chelators is important in achieving this goal, other factors such as psychological wellbeing, family and institutional support also impact on adherence and outcomes.

In this chapter we first describe the effects of iron overload and the tools for monitoring excess iron. We then cover the general goals of chelation therapy, and the mechanisms by which chelators work. Recommendations for the dosing of three licensed chelators are then described, based on evidence on their efficacy. The potential toxicities of each chelation regime and how to minimise their risks are given in **Appendix 1**. Finally, guidelines for monitoring chelation therapy so as to minimize the risks of toxicity from iron chelation are discussed.

The Rate of Iron Loading

Blood transfusion

Gaining the most accurate information on the rate of iron loading from transfusion therapy is important in assisting selection of the best chelation therapy for each patient. A unit processed from 420 mL of donor blood contains approximately 200 mg of iron, or 0.47 mg/mL of whole donor blood. For red cell preparations with variable haematocrits, the iron per mg/mL of blood can therefore be estimated from 1.16 x the haematocrit of the transfused blood product. In cases where organizational systems or other difficulties prevent such estimations to be calculated, a rough approximation can be made based on the assumption that 200 mg of iron is contained in each donor unit. Irrespective of whether the blood used

is packed, semi-packed or diluted in additive solution, if the whole unit is given, this will approximate to 200 mg of iron intake. According to the recommended transfusion scheme for thalassaemia major (**Chapter 2**), the equivalent of 100–200 ml of pure red blood cell (RBC) per kg body weight per year are transfused. This is equivalent to 116-232 mg of iron/kg body weight / year, or 0.32-0.64 mg/kg/day. Regular blood transfusion therapy therefore increases iron stores to many times the norm unless chelation treatment is provided. If chelation therapy is not given, **Table 1** shows how iron will accumulate in the body each year, or each day.

Table 1. Iron loading rates in the absence of chelation.

PATIENTS WEIGHT	20 kg	35 kg	50 kg	65 kg
Pure red cells vol. ml/year	2,000-4,000	3,500-7,000	5,000-10,000	6,500-13,000
Yearly iron loading (g)	2.3-4.6	4.1-8.2	5.8-11.6	7.5-15.1
Daily Iron loading (mg)	6.3-12.6	11.2-22.5	15.9-31.8	20.5-41.4

Increased gastro-intestinal absorption of iron

In transfusion dependent thalassaemia (TDT), the contribution of iron absorbed from the diet is small compared with blood transfusion. Normal intestinal iron absorption is about 1-2 mg/day. In patients with thalassaemia who do not receive any transfusion, iron absorption increases several-fold. It has been estimated that iron absorption exceeds iron loss when expansion of red cell precursors in the bone marrow exceeds five times that of healthy individuals. Transfusion regimens aimed at keeping the pre-transfusion haemoglobin above 9 g/dl have been shown to prevent such expansion (Cazzola 1997). In individuals who are poorly transfused, absorption rises to 3-5 mg/day or more, representing an additional 1-2 g of iron loading per year.

Toxicity from Iron Overload

Mechanisms of iron toxicity

Iron is highly reactive and easily alternates between two states – iron III and iron II – in a process which results in the gain and loss of electrons, and the generation of harmful free radicals (atoms or molecules with unpaired electrons). These can damage lipid membranes, organelles and DNA, causing cell death and the generation of fibrosis. In health, iron is 'kept safe' by binding to molecules such as transferrin, but in iron overload their capacity to bind iron is exceeded both within cells and in the plasma compartment. The resulting 'free iron', either within cells or within plasma, damages many tissues in the body or is fatal unless treated by iron chelation therapy. Free iron also increases the risk of infections **(Chapter 7)** and neoplasia. A summary of the mechanisms for toxic effects of iron overload is shown in **Figure 1.**

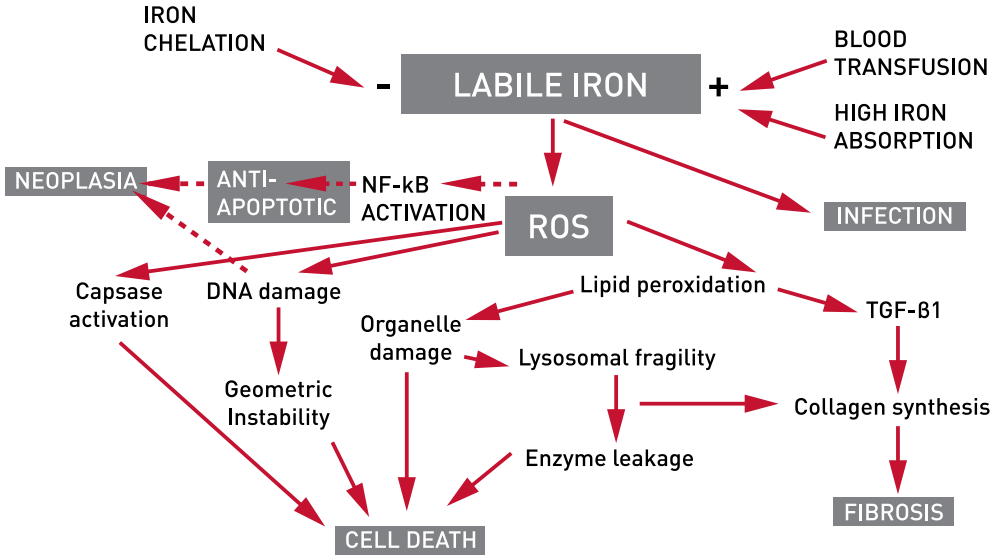

Figure 1. Pathological mechanisms and consequences of iron overload. In iron overload resulting from repeated blood transfusions or long-term increased iron absorption, iron that is not bound to naturally occurring molecules such as transferrin, or ferritin or to therapeutic iron chelators, generates a variety of reactive oxygen species (ROS), most notably hydroxyl radicals. This occurs in cells where labile plasma iron is taken up and accumulated as storage iron (ferritin and haemosiderin). ROS generate lipid peroxidation, organelle and DNA damage and dysregulate mechanisms involved in apoptotic cell death, increasing the risk of neoplasia such as hepatoma. Labile iron is also more available to microorganisms that iron bound to transferrin or ferritin, thereby increasing the risk of infection. Reproduced with permission from (Porter 2014).

Distribution and consequences of transfusional iron overload

In the absence of iron overload, uptake of iron into cells is controlled by the interaction of transferrin with its receptors - mainly on red cell precursors, hepatocytes and dividing cells. In iron overload, transferrin becomes saturated and iron species that are not bound to transferrin are present in plasma (plasma non transferrin bound iron, or NTBI). The distribution of NTBI uptake is fundamentally different from transferrin uptake, and is thought to involve calcium channels. Organ damage in transfusional iron overload reflects the pattern of tissue iron uptake from NTBI. Some tissue are spared from iron loading through this mechanism (such as skeletal muscle), while other such myocardial muscle, endocrine tissue and hepatocytes take up NTBI rapidly. This iron is then stored as ferritin or haemosiderin which are visible by MRI. The myocardial iron overload induces heart failure from cardiomyopathy in patients without chelation in as early as the second decade of life. Iron overload also causes pituitary damage, leading to hypogonadism, growth retardation and delayed puberty. Endocrine complications, namely diabetes, hypothyroidism and hypoparathyroidism are also seen. Liver disease with fibrosis and eventually cirrhosis and hepatocellular carcinoma, particularly if concomitant chronic hepatitis is present, are also serious complications (see **Chapter 5**).

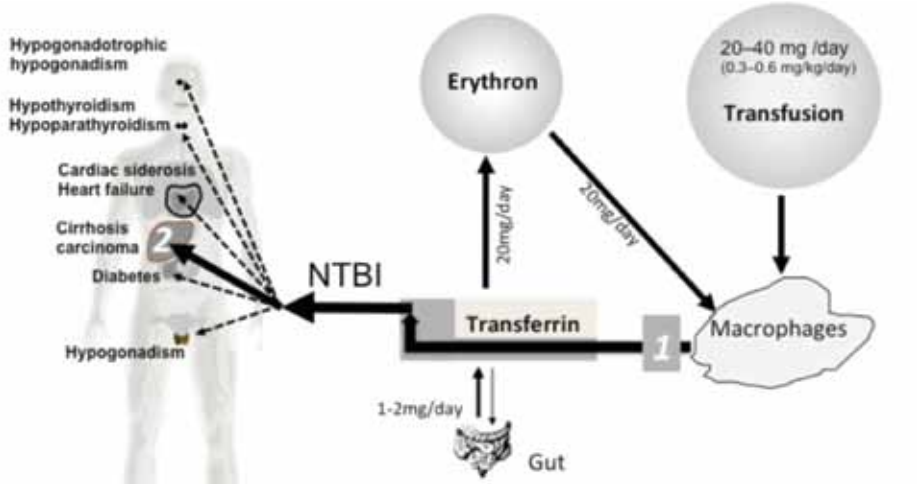

Figure 2. The main routes of iron turnover and uptake are shown by solid black arrows on the right panel: 20 mg of iron is delivered daily to the erythron in health. This increases several fold in untransfused thalassaemias but can be inhibited by hypertransfusion. NTBI is generated when transferrin (which is about 30% saturated in healthy adults) becomes saturated. Transferrin saturation occurs either following iron overload of the macrophage system, but also as a result of decreased clearance of transferrin iron in hyper-transfused patients. The organs in which NTBI are taken up and retained as storage are shown on the left, with >80% cleared by hepatocytes. Despite variable and low lower quantities of iron taken into other tissues (represented by broken lines), serious and often irreversible iron-mediated damage may occur. Iron excretion by chelation therapy acts mainly at sites (1): the interception of iron released from macrophages after red cell catabolism, and (2): iron released by the catabolism of ferritin within hepatocytes.

Monitoring of Iron Overload

Monitoring is essential in establishing effective iron chelation regimes, tailored to individuals' specific needs. However, some general principles of monitoring iron overload apply to all.

Serum ferritin

Why measure serum ferritin?

Serum ferritin (SF) generally correlates with body iron stores, and is relatively easy and inexpensive to determine repeatedly. Serum ferritin is most useful in identifying trends. A decreasing trend in SF is good evidence of decreasing body iron burden but absence of a decreasing trend does not exclude a decreasing iron burden. However, an increasing SF trend implies an increasing iron burden but may also be due to inflammation or tissue damage, so clinical judgment must be used to interpret these trends. Long term control of SF is also a useful guide to the risk of complications from iron overload in TM; many studies have shown an association between the control of serum ferritin and prognosis (Borgna-Pignatti 2004, Davis 2004, Gabutti 1996, Olivieri 1994). Studies have identified a significantly lower risk of cardiac disease and death in at least two-thirds of cases where serum ferritin levels have been maintained below 2,500 µg/L (with Deferoxamine, or DFO) over a period of a decade or more (Olivieri 1994). Observations with larger patient numbers show that maintenance of an even lower serum ferritin of 1,000 µg/L may be associated with additional clinical advantages (Borgna-Pignatti 2004) (see **Table 2)**.

What are the limitations of serum ferritin measurements?

Most SF assays were developed mainly for detecting iron deficiency, and the linear range of the assay at high SF values needs to be known. SF must be performed in a laboratory that has established how to dilute samples with high values, to give readings within the linear range of the assay. SF measures do not always predict body iron or trends in body iron accurately. In TM, variation in body iron stores accounts for only 57% of the variability in plasma ferritin (Brittenham 1993). This variability is in part because inflammation increases serum ferritin, and partly because the distribution of liver iron between macrophages (Kupffer cells) and hepatocytes in the liver has a major impact on plasma ferritin. A sudden increase in serum ferritin should prompt a search for hepatitis, other infections, or inflammatory conditions.

A lack of fall in SF with chelation does not therefore necessarily prove that the patient is a 'non responder' to the chelation regime. As outlined above, this can be because inflammation may have falsely raised SF, or because the relationship between body iron and SF is not always linear, particularly in the context of inflammation or tissue damage (Adamkiewicz 2009), and body iron can fall considerably from a high starting point (e.g. LIC >30 mg/g dry wt) before a change in ferritin is clear. Below 3000 µg/L SF values are influenced mainly by iron stores in the macrophage system, whereas above 3000 µg/L they are determined increasingly by ferritin leakage from hepatocytes (Davis 2004, Worwood 1980). Day-to-day variations are particularly marked at these levels. The relationship between serum ferritin and body iron stores may also vary depending on the chelator used (Ang 2010) and by duration of chelation therapy (Fischer 2003).

Table 2. Use of SF for monitoring chelation treatment.

ADVANTAGES	DISADVANTAGES
Easy to assess repeatedly	Indirect estimate of iron burden
Inexpensive	Increased by inflammation
Trend identification possible with repeat samples	Cannot determine iron balance directly
Long term control linked to outcome	Non-linear response to iron load at high levels
Useful for dose adjustment as iron levels fall	Absence of decrease doesn't exclude response
	Relationship to iron load varies with chelator
	Relationship to LIC differs in different diseases

Liver iron concentration (LIC) measurement

Uses for liver iron concentration monitoring

- **To identify whether body iron is adequately controlled.**
  Adequate control of LIC is linked to the risk of hepatic damage as well as the risk of extrahepatic damage. Normal LIC values are up to 1.8 mg/g dry wt, with levels of up to 7 mg/g dry wt seen in some non-thalassaemic populations without apparent adverse effects. Sustained high LIC (above 15-20 mg/g dry wt) have been lined to worsening prognosis, liver fibrosis progression (Angelucci 1997) or liver function abnormalities (Jensen 2003). In the absence of prior iron chelation therapy, the risk of myocardial iron loading increases with the number of blood units transfused and hence with iron overload (Jensen 2003. Buja 1971). However, the relationship between LIC and extra-hepatic iron is complicated by chelation therapy as iron tends to be accumulate initially in the liver and later in the heart but also is removed more rapidly from the liver than the heart by chelation therapy (Noetzli 2008, Anderson 2004). Thus in patients receiving chelation therapy, whilst high LIC increases the risk of cardiac iron overload, the measurement of LIC will not predict myocardial iron and hence cardiac risk reliably, and myocardial iron may be found in some patients despite currently well controlled LIC.

- **To determine iron balance: is body iron increasing or decreasing on current therapy?**
  LIC is the most reliable indicator of body iron load, which can be derived from the following formula: Total body iron stores in mg iron /kg body wt = 10.6 x the LIC (in mg/g dry wt) (Angelucci 2000). Sequential measurement of LIC is the best way to determine whether body iron is increasing or decreasing with time (iron balance). While serum ferritin is simple, relatively inexpensive and can be repeated frequently, LIC determination should be considered for those patients whose serum ferritin levels deviate from expected trends (i.e. those with suspected co-existing hepatitis, or patients on chelation regimens with variable or uncertain responses), as this may reduce the risk of giving either inadequate or excessive doses of chelation therapy. Since the relationship of SF to iron overload and iron balance has not yet been established, assessment of LIC may be particularly useful when new chelating regimes are being used. At high levels of SF (>4000 µg/L), the relationship to LIC is not linear and patients may show fall in LIC (negative iron balance) without a clear trend in SF in the first 6-12 months. When a patients fails to show a fall in SF over several months the change in LIC can identify whether the current regime is adequate or need to be modified (increased frequency or adherence, increased dose, or change in regime).

Methods for measuring LIC

- **Biopsy**
  Measurement of LIC was initially done by chemical determination on a liver biopsy sample (fresh, fixed or from dewaxing of paraffin-embedded material) (see Table 3). Biopsy is an invasive procedure, but in experienced hands has a low complication rate (Angelucci 1997). Inadequate sample size (4 mg dry wt or about a 2.5 cm core length) or uneven distribution of iron, particularly in the presence of cirrhosis (Villeneuve 1996), may give misleading results however. Biopsy also allows the evaluation of liver histology which cannot yet be reliably estimated by non-invasive means. Laboratory standardization is not trivial and differences between laboratories, for example in wet

to dry weight ratios, can mean that results from different labs may not be equivalent.

- **SQUID**
  Magnetic biosusceptometry (SQUID) (superconducting quantum interference device) determines the paramagnetism of the liver which is proportional to LIC (Brittenham 1993). Current methodology requires liquid helium which is very expensive. Furthermore, the SQUID apparatus needs to be in an environment away from paramagnetic forces (e.g. lifts, cars) which is often impractical. For these reasons, the current generation of SQUID devices are unlikely to be used outside a small number of well-resourced centres. Surprisingly not all SQUID devices have been calibrated the same, so comparison of results from different centres must be interpreted with caution unless the relevant machines have been cross-validated.

- **MRI**
  MRI techniques are now becoming the most widely used methods for LIC determination. The first techniques compared the signal in the liver or heart with that of skeletal muscle, which does not accumulate iron (Jensen 1994). However, this is not widespread use today and has been superseded by better methods. The principle shared by all MRI techniques currently used is that when a radio-frequency (rf) magnetic field pulse is applied to the tissue (e.g. liver or myocardium), protons take up energy, altering their spin orientation and they later relax returning to their original state. With spin echo, after the pulse the nuclei take time to relax in the "relaxation time"; T1 in the longitudinal plane, and T2 in the transverse plane. Values may also be expressed as relaxation rates, the R1 rate (is the same as 1/T1) and the R2 rate (is the same as 1/T2). A variation of this principle are Gradient Echo techniques, achieved by applying a strong graded magnetic field to the radio-frequency (rf) pulse that is used for spin echo. This relies on multiple echoes over a shorter acquisition time period than spin echo techniques. The shorter acquisition may improve sensitivity and can be measured as T2* (in ms), where 1/T2* = 1/T2 + 1/T2', and T2 is the tissue relaxation time and T2' is the magnetic inhomogeneity of the tissue. An important point is that tissue iron concentration is not linearly related to T2* or the T2, but is linearly related to 1/T2* or 1/T2 (R2* or R2). Both gradient and/or spin echo techniques have been used in clinical practice. T2* (or R2*) can be achieved with a single breath hold, while T2 or R2 take a little longer to acquire data. Manufacturers of suitable MRI scanners are: Siemens (Erlangen, Germany); GE Healthcare (Milwaukee, WI, USA); Philips Healthcare. The strength of the magnetic field applied by these scanners is measured in Tesla (T) units. Most imaging is done on 1.5T machines but 3T machines give a better signal to noise ratio. However, 3T machines have greater susceptibility to artefacts, and the maximum detectable iron level is also halved (which is too low for many patients) (Wood 2008, Storey 2007). At present only 1.5T machines are widely used with reliable precision and accuracy based on standardised validation procedures. Liver packages (including standard sequences and analysis of the data) are included in the software provided with these MRI machines. Specialized LIC analysis software can also be bought separately.

  A note of caution is that the different MRI techniques may not be equivalent – at least in the manner they are currently calibrated and practiced. The first widely used technique was the T2* technique (Anderson 2001), where liver biopsy was used to calibrate the method. Although this demonstrated the principle of T2* to measure liver iron, unfortunately due to factors such as long echo times (TE 2.2-20.1 ms), and multi breath-hold acquisition,

the calibration differs from later techniques, and can underestimate LIC by two-fold. Therefore studies using this calibration may underestimate LIC (Garbowski 2009). The R2 based Ferriscan technique appears to have acceptable linearity and reproducibility up to LIC values of about 30 mg/g dry wt (St Pierre 2005), with an average sensitivity of >85% and specificity of >92% up to an LIC of 15 mg/g dry wt, and has been registered in the EU and US. For calibration of Ferriscan, the MRI machine must use a Phantom supplied by the company, while the data acquired is sent via internet for analysis by dedicated Ferriscan software (payment per scan analyzed). A particular advantage of this technique is that it can be applied with little training, at any centre with a reasonably up-to-date MRI machine (see **Table 3**).

Table 3. Rationale, advantages and disadvantages of LIC determination by MRI and biopsy.

ADVANTAGES	DISADVANTAGES
Gives the most reliable estimate of body iron	Expensive (either by biopsy or MRI)
Allows calculation of iron balance (LIC change)	Cannot be repeated as frequently as SF (cost with MRI or inconvenience with biopsy)
Long term LIC control - linked to prognosis	LIC unreliable as predictor of heart iron in chelated patients
LIC not affected by inflammation (unlike SF)	Biopsy risks complications (low in expert centre)
Biopsy shows degree of liver damage	Biopsy method affected by sampling artifact
MRI non-invasive with good patient acceptance	MRI method is not universally available
MRI method can readily be set up and stardardised across different centres	MRI method requires external validation
	MRI determination unreliable above LIC of 30 mg/g dry wt

Myocardial iron estimation: T2* and other tools

The physical principles of iron measurement for the heart by MRI are the same as for the liver (see above), with the additional challenge of measuring a moving object - the myocardium. The T2* (or R2*) techniques have the advantage over T2 or R2 in that they require shorter acquisition times and can be achieved with a single breathold (Kirk 2010). The utility of myocardial T2* (mT2*) MRI was originally identified on the basis of shortened T2* values <20 ms in patients with decreased left ventricular ejection fraction (LVEF) (Anderson 2001). More recently the relationship between biochemically measured myocardial iron concentration

and myocardial T2* has been shown using post mortem myocardial material (Carpenter, 2011). Here, mean myocardial iron causing severe heart failure in 10 patients at post mortem was 5.98 mg/g dry weight (ranging from 3.2 to 9.5 mg/g); levels that in the liver would not be regarded as harmful to the liver. The relationship of myocardial iron concentration (MIC) to T2* is: MIC (mg/g dry wt) = 45 * (T2* ms)^-1.22 (Kirk 2009b). This relationship is non-linear so small changes in mT2* at values <10 ms may indicate relatively large changes in MIC. The risk of developing heart failure increases with T2* values <10 ms, which are associated with a 160 fold increased risk heart failure in the next 12 months (Kirk 2009b). This risk further increases progressively with T2* values <10 ms, so that the proportion of patients developing heart failure in the next 12 months at T2* of 8-10 ms, 6-8 ms and <6 ms was 18%, 31%, and 52% respectively. These risks were derived from patients whose chelation therapy and adherence was not reported, so this risk may be less in patients taking regular chelation. For example, in a recent prospective study in patients with severe myocardial iron loading (T2* values <10 ms), no patients developed heart failure over a 2 year period while taking deferasirox (DFX) and desferrioxamin (DFO) combination therapy (Ayidinok 2014).

In centres where the T2* method has been validated, the T2* value may have predictive value in identifying patients at high risk of developing deterioration in LVEF, thus allowing targeted intensification of treatment before heart failure develops. The value of T2* monitoring is supported by a recent report in a cohort of TM patients monitored for 10 years using T2*, in which iron mediated cardiomyopathy was no longer the leading cause of death, and the proportion of patients with T2* <20 ms fell from 60% to 1% over the decade (Thomas 2010). Alternative factors such as improved chelation options may also have contributed to these improvements in outcome. T2* monitoring has now been established and validated internationally (Kirk 2010), and is now recommended as part of yearly monitoring of multi-transfused patients at risk of developing myocardial iron loading. However it is very important that the method adopted in a given centre undertakes measurements to independently validate and calibrate measurements, otherwise inappropriate assessment of heart failure prognosis may result. **Table 4** summairzes advantages and disadvantages of using T2* MRI for monitoring cardiac iron overload.

Heart function

Sequential monitoring of LVEF has been shown to identify patients at high risk of developing clinical heart failure (Davis 2004, Davis 2001). When LVEF fell below reference values, there was a 35 fold increased risk of clinical heart failure and death, with a median interval to progression of 3.5 years, allowing time for intensification of chelation therapy. This approach required a reproducible method for determination of LVEF (such as MUGA or MRI), while echocardiography was generally too operator-dependent for this purpose. Furthermore, there is a clear need to identify high risk patients before there is a decline in LVEF. Myocardial T2* by MRI can achieve this and has additional predictive value (see above). However, as only a subset of patients with T2* values between 10 and 20 ms, or even with T2* less than 10 ms have abnormal heart function, sequential measurement of LVEF can identify the subset of patients who have developed decompensation of LV function and are therefore at exceptionally high risk and require very intensive chelation therapy (see below).

Table 4. MRI T2* method to assess myocardial iron.

ADVANTAGES	DISADVANTAGES
Rapidly assessed iron content in myocardial septum	Indirect non-linear relationship with myocardial iron
Reproducible method	Requires a validated centre with dedicated methods
Linked to heart iron (reciprocal relationship)	Technically demanding
Potential to measure heart function at same visit	Methodology requires standardisation worldwide
Potential to measure LIC at same visit	Does not predict liver body iron overload
Linked to LVEF at time of measurement	Requires continuous quality assurance such as regular phantom scanning
Linked to risk of heart failure in next year	

Monitoring of other organ function and iron mediated damage

The monitoring of organ function as a marker of damage from iron overload is discussed more fully in other chapters. In general by the time diabetes, hypothyroidism, hypoparathyoidism or hypogonadotropic hypogonadism (HH) have been identified, irreversible damage has set in and the focus then becomes replacing hormones. These are late effects and the primary aim of chelation therapy is to prevent such damage. Iron overloaded patients should be monitored for evidence of hypogonadotropic hypogonadism (growth and sexual development and biochemical markers of HH), diabetes mellitus (yearly OGTT), hypothyroidism and hypoparathyroidism. There has been recent interest in using MRI as a way of identifying the risks of iron-mediated damage to the endocrine system. Early work in this area showed good correlation between MRI findings (loss of pituitary volume) and biochemical markers of pituitary damage (Chatterjee 1998). With improved MRI imaging, other endocrine organs have also been evaluated (Wood 2007). It is of interest that there is generally a close correlation between iron deposition in the heart and deposition in endocrine tissues such as those in the pituitary and pancreas (Noetzli 2009, Au 2008). This supports the notion of shared uptake mechanisms for NTBI in heart and endocrine systems and supports clinical observations of shared risks in cardiac and endocrine systems once iron begins to escape from the liver.

24h Urinary iron estimation

Measurement of the urinary iron excretion has been used in assessing the effect on iron excretion by desferioxamine (about half of total iron excreted in urine) (Pippard 1982) or deferiprone (over 80% of iron excreted in urine), but is not useful in patients treated with

deferasirox, as nearly all the iron is excreted in faeces. Urine iron has also been used to compare effects of combination and monotherapy regimes containing deferiprone (DFP) (Aydinok 2012a, Mourad 2003). The inherent variability in daily iron excretion necessitates repeated determinations and this is not widely used in routine monitoring

Plasma non-transferrin bound iron and labile plasma iron

As plasma iron that is unbound to transferrin (NTBI) is considered to be the main route through which iron is distributed to liver and extrahepatic targets of iron-overloaded thalassaemia major patients, levels of NTBI might be expected to correlate with the risk of damage to these tissues. Assays may estimate NTBI directly using a chelation capture method followed by HPLC (Singh 1990), or by colorimetric analysis (Gosriwatana 1999) or indirectly by exploiting the impact of labile iron species to oxidised fluochrome, such as in the labile plasma iron (LPI) assay (Zanninelli 2009, Cabantchik 2005). A potential advantage of the LPI assay is that it is better suited to measurements when iron chelators are present in the plasma (Zanninelli 2009). Whilst some loose associations of NTBI (Piga 2009) or LPI (Wood 2011) with some markers of cardiac iron or response to chelation have been found by some investigators, thus far measurements have not been sufficiently strongly predictive of cardiac risk to be recommended for routine clinical practice. This is partly because NTBI and LPI are highly labile, rapidly returning or even rebounding (Porter 1996) after an iron chelator has been cleared (Zanninelli 2009). Although NTBI correlates loosely with iron overload, it is affected by other factors such as ineffective erythropoiesis, the phase of transfusion cycle, and the rate of blood transfusion (Porter 2011) adding to the complexity of interpreting levels (Hod 2010). It is also not clear which methods identify the iron species that are most strongly inked to myocardial iron uptake. Therefore although the measurement of NTBI (or LPI) has proved a useful tool for evaluating how chelators interact with plasma iron pools, its value as a guide to routine treatment or prognosis has yet to be clearly demonstrated.

Treatment of Iron Overload

Aims of iron chelation therapy

1) **Prevention therapy:**
   The primary goal of chelation therapy is to maintain safe levels of body iron at all times, by balancing iron intake from blood transfusion with iron excretion by chelation (iron balance).

2) **Rescue therapy:**
   Once iron overload has accumulated, more iron must be removed than accumulates as a result of blood transfusion. Removal of storage iron is slow and inefficient, because only a small proportion of body iron is available for chelation at any given time. Once iron has deposited in some tissues, damage is often irreversible. Prevention is therefore preferable to rescue therapy. Chelation therapy should therefore be initiated before toxic levels of iron have accumulated.

3) **Emergency therapy:**
   If heart failure develops urgent action is required, which usually requires changing and/or intensifying the treatment.

4) **Dose adjustment of therapy:**
Dosing and treatment regimens require adjustment to changing circumstances. These can be identified by careful monitoring of iron and its distribution. Without monitoring of trends in iron load (liver iron and ferritin) and iron distribution (heart iron and function) patients are at risk of either a) underchelation with increased iron toxicity; or b) overchelation and increased chelator toxicity. The dosing and regime must be adjusted periodically to take these factors into account.

5) **Adherence to therapy:**
Chelation must be taken regularly for it to work effectively. This requires good adherence to the chelation regime. Intermittent high dose chelation can induce negative iron balance but does not provide continuous protection from labile iron and risks increased toxicity from the iron chelator. Poor adherence can result from practical issues such as difficulty with DFO infusions, intolerance of a particular chelator, or from psychological / psychosocial issues. A key role of the treating centre is the monitoring and encouragement of adherence to chelation, alongside support from their family. However, encouraging a patient to take control or 'self-manage' is often a useful approach of long-term benefit.

Sources of chelatable iron

Only a very small fraction of body iron is available for iron chelation at any moment of time. This is because iron chelators interact with low molecular weight 'labile' iron pools better than with iron stored as ferritin or haemosiderin. Labile iron is constantly being generated, so that the efficiency of chelation is better when a chelator is available at all times (chelator present 24 hours a day). 24h chelation also has the potential to remove toxic labile iron pools within cells continuously, which is particularly important in reversing heart failure. Chelatable iron is derived from two major sources: iron derived from the breakdown of red cells in macrophages (about 20 mg/day in healthy adults), and iron derived from the catabolism of stored ferritin iron within cells. Most of the storage iron in the body is in hepatocytes, and the ferritin in these cells is turned over less frequently (every few days). Iron chelated within the liver is excreted though the biliary system, or circulates back into plasma and is excreted in the urine. The extent to which this chelated iron is eliminated in faeces or urine varies with each chelator. With DFO about half is excreted in urine and half in faeces, whilst with DFX excretion is mainly through the urine and DFP through faeces. Urinary excretion of iron chelated by DFO is derived mainly from macrophage catabolism of red cells, whereas urine iron chelated by DFP is derived from macrophage and hepatocyte pools. Small quantities of storage iron are also deposited in the endocrine system and in the heart. Because these are not designed as cells for iron storage and release, unlike hepatocytes, storage iron is turned over in the lysosome compartment less frequently and a lower proportion of cellular iron is available for chelation at any moment. Thus it generally takes longer to remove iron from these tissues than from the liver.

Chemical and pharmacological properties of licensed chelators

Three iron chelators are currently licensed for clinical use and their iron binding properties, routes of absorption, elimination and metabolism differ. These are summarized in **Table 5**. Of note, the majority of information presented refers to prototype formulations of the chelators.
Chemistry: The number of chelator molecules required to bind iron differs with each of

these chelators. DFO binds iron in a 1:1 ratio, which results in a very stable iron chelate complex but also a large molecule that cannot be absorbed from the gut. DFX binds iron in a 2:1 chelator to iron ratio, and is small enough for oral absorption. DFP is smaller still and requires 3 molecules to bind iron, resulting in a less stable iron complex and a lower efficiency of iron binding at low chelator concentrations (low pM).

Pharmacology: The patterns of elimination of the chelate-iron complexes are shown in **Table 5.** Iron free DFO is eliminated rapidly in urine and faeces (short T ½) if it does not bind iron, but the elimination of iron complexes are slower. Iron free DFP has a short plasma half-life, requiring it to be given 3 times a day. It is rapidly metabolized at its iron binding site in hepatocytes. DFX has a longer plasma half-life, typically requiring only once daily dosing and providing 24 clearance of labile plasma iron. Plasma drug levels differ between the chelators. DFO levels rarely exceed 10 μM when given as an infusion at night, and negligible levels of iron-free chelator are present during the day. DFP levels fluctuate with peaks exceeding 100 μM at approximately 2h after ingestion but with negligible levels at night, if the 3 doses are given during the day (Aydinok 2012a, Limenta 2011). DFX and its iron complex are eliminated in faeces (**Table 5**) (Nisbet-Brown 2003), and about 10% of plasma DFX is bound to iron (Galanello 2003). Metabolism is mainly by glucuronidation to iron binding metabolites, with less than 10% of metabolism being oxidative, by cytochrone p450 (Waldmeier 2010).

Table 5. Chemical and pharmacological properties of licensed chelators.

COMPOUND	Desferrioxamine (DFO)	Deferasirox (DFX)	Deferiprone (DFP)
Molecular weight (daltons)	560	373	139
Log Iron binding affinity (pM)	26.6	22.5	19.9
Delivery	s.c.or i.v. 8-12 hours 5 days/week	Oral, once daily	Oral, 3 times daily
Half-life of iron free drug	20-30 minutes	12-16 hours	3-4 hours
Lipid solubility	Low	High	Intermediate
Route of iron excretion	Urinary and faecal	Faecal	Urinary
Max plasma levels (μM) of iron free drug	7-10 (Porter 2005b)	80 (Galanello 2003)	90-450 (Kontoghiorghes 1990)
Concentration of iron complex	Complex remains similar (about 7 μM) with ascending doses but the iron-free drug and metabolites increase (Porter 2005b)	Complex accounts for about 10% of plasma drug in steady (Waldmeier 2010)	Complex correlates with urine iron excretion and predicts response to therapy (Aydinok 2005)

COMPOUND	DESFERRIOXAMINE (DFO)	Deferasirox (DFX)	Deferiprone DFP)
Min. plasma level (μM) With daily dosing	0	20	0
Elimination of iron complex	Urine + faeces Iron complex removed more slowly than free drug	Faeces	Urine
Metabolism	Intrahepatic to metabolite B which binds iron (Porter 2005b, Porter 1998)	>90% eliminated in faeces, 60% unmetabolised. Metabolism mainly in liver to glucuronides. Oxidative metabolism by cytochrome 450 accounts for < 10%. Most metabolites bind iron (Waldmeier 2010)	Glucuronide formed in liver does not bind iron (Kontoghiorghes 1990)
Recommended dose mg/kg/d	30-60 5-7 x/week	20-40 once daily	75-100 in 3 divided doses
Chelation efficiency (% of drug excreted iron	13	27	7
Main Adverse effects (For more see details Appendix 2)	Ocular, auditory, bone growth retardation local reactions, allergy	Gastrointestinal, increased creatinine, increased hepatic enzymes	Gastrointestinal, arthralgia, agranulocytosis/ neutropenia

Practical prescribing of individual chelators

In general, as with any therapy, the potential benefits of chelation therapy must be balanced against occasional unwanted adverse effects which are generally more likely when doses are high relative to the level of iron overload. These typically take time to develop, so that careful monitoring should reduce these risks. Unfortunately the combination of chelation therapies is not specifically licensed, so there is no prescribing information provided by licensing authorities in this respect. However, the clinical and research experience with combination therapies will be described as are used in many treatment centres when monotherapy is inadequate. Appendix 1 summarises the particular prescribing information from licensing authorities which act as a guide for prescribing individual monotherapies.

Desferrioxamine monotherapy (Desferal® or deferoxamine; DFO)

DFO is licensed for the treatment of chronic iron transfusional iron overload worldwide for affected patients above the age of 2 years, reflecting its long-standing clinical use. There

are some small differences in age of treatment commencement and maximum doses recommended in different countries.

Evidence of beneficial effects

DFO was the first chelator introduced clinically. A large body of literature has since emerged on the changing complications and improved survival, predominantly from retrospective cohort analysis. As no treatment alternatives were available at the time of its introduction, the benefits of its long term use are clearer than for newer chelators, where patients have often received more than one chelation treatment over a lifetime. The main disadvantages of the treatment are that it is costly and it must be administered parenterally which is uncomfortable and time consuming. Also because of its short half live it typically only chelates iron during the time infused, therefore leaving 12 or more hours with no active chelator with standard regimens. The increased toxicity of DFO at low levels of body iron (see **Appendix 2**) means that guidelines for its use have been conservative, generally recommending that therapy not be started until SF levels reach 1000 µg/L, and with care to avoid over-chelation below this SF value.

Effects on serum ferritin

Long term control of SF has been linked to protection from heart disease and to improved survival if levels are consistently less than 2500µg/L (Olivieri 1994) with even better outcomes at levels <1000µg/L (Borgna-Pignatti 2004). Four decades of clinical experience clearly show that ferritin can be controlled with DFO monotherapy at 40-50 mg/kg administered as an 8-10-h infusion at least 5 times a week. In children however, mean daily doses should not exceed 40 mg/g because of the effects on growth and skeletal development. Guidelines about the dosing required to control iron overload were based on retrospective data until recently. A randomised study in 290 TM patients identified the doses required to stabilize or decrease SF, with a mean daily dose of 42 mg/kg resulting in a small decrease in serum ferritin of 364µg/L at one year, whereas 51 mg/kg resulted in an average decrease of approximately 1,000 µg/L (Cappellini 2006). Further analysis shows that response is also linked to the transfusion rate and that larger doses are required in patients requiring higher transfusions (see below) (Cohen 2008). Thus the effectiveness of DFO at controlling SF is related to dose, frequency and duration of exposure and transfusion rate.

Effects on liver iron

Administered at least 5 times a week in sufficient doses, DFO is effective in controlling liver iron and hence total body iron stores (Brittenham 1993). In a prospective randomized study (Cappellini 2006), a mean dose of 37 mg/kg stabilised LIC for patients with baseline LIC values of between 3 and 7 mg/g dry wt. For patients with LIC values between 7 and 14 mg/g dry wt, a mean dose of 42 mg/kg resulted in a small decrease of 1.9 mg/kg dry wt over a 1 year interval. In patients with LIC values >14 mg/g dry wt, a mean dose of 51 mg/kg resulted in LIC decreases of an average of 6.4 mg/g dry wt. Thus a dose of 50 mg/kg at least 5 days a week (giving a mean daily dose of 50 x 5/7 = 36 mg/kg) is recommended if a significant decrease in optimal LIC levels is required (see above). It should be emphasised that these are average changes and that the dose required may increase or decrease depending on transfusion requirements (Cohen 2008).

Effects on heart function

Subcutaneous therapy has long been known to prevent (Wolfe 1985) or improve asymptomatic cardiac disease in thalassaemia major (Aldouri 1990, Freeman 1983). After the introduction

of DFO, the incidence of iron- induced heart disease in different cohorts of patients fell progressively – with a key factor being the age of starting treatment (Borgna-Pignatti 2004, Brittenham 1994). Symptomatic heart disease can be reversed by high dose intravenous treatment (Davis 2000, Cohen 1989, Marcus 1984). The same results can be obtained with excellent long-term prognosis with lower doses (50-60 mg/kg/day – see below), and consequently less drug toxicity using continuous dosing (Davis 2004, Davis 2000). Continuous intravenous doses of 50-60 mg/kg/day typically normalise LVEF in a period of three months (Anderson 2004), significantly before liver or heart iron stores have been normalised. However, if advanced heart failure has developed before treatment is intensified, the chances of successful rescue are reduced. Early intervention for decreased LV function is therefore recommended. Once heart function has improved, sustained compliance is critical to improve outcomes, especially while myocardial iron remains increased (Davis 2004).

Effects on heart iron (mT2*)

Myocardial iron can improve with either subcutaneous or intravenous therapy provided treatment is given in adequate doses and frequency. Improvement in mild to moderate cardiac T2*, even at low intermittent doses (5 days a week) has been confirmed by prospective randomised studies (Pennell 2014, Pennell 2006b, Tanner 2006). For patients with established mild to moderate myocardial iron, a simple increase in dose or frequency of use may be sufficient to improve the mT2*. For example at relatively low doses of 35 mg/kg, an average improvement in T2* of 1.8 ms over one year has been shown (Pennell 2006b). At a slightly higher dose of 40-50 mg/kg five days a week, patients showed an improvement of 3 ms over one year (Porter 2005a). When mT2* is < 10 ms, as with other iron chelators, it will take several years of sustained and compliant therapy to normalise myocardial iron (Porter 2002). For T2* values <10 ms, a simple 5 day a week s.c. DFO at standard doses is unlikely to be sufficient, and treatment intensification is indicated. This could involve higher dose continuous DFO or more likely switching to another chelation regime in the absence of heart failure (see below).

Effects on long term outcome

DFO has been in clinical use since the 1970s and widely used as subcutaneous infusions since about 1980. The most powerful evidence for the effectiveness of DFO and indeed for chelation as a treatment modality is the improving survival and decreased morbidity in patients treated with DFO since this time (**Table 6**). This benefit is clearly shown in successive cohorts born since this time. Only patients born after 1980 will have started treatment at an early age, and age of starting treatment is a key factor in outcome (Borgna-Pignatti 2004, Brittenham 1993). Regular subcutaneous therapy started before the age of 10 years reduces co-morbidities such as the incidence of hypogonadism (Bronspiegel-Weintrob 1990), as well as other endocrine disturbances, including diabetes mellitus (Borgna-Pignatti 2004, Olivieri 1994, Brittenham 1993). Adherence to therapy has been the main limiting factor to successful outcomes; failure to take treatment at least 5 times a week at adequate doses and subsequent failure to control serum ferritin in the long term leads to increased mortality (Gabutti 1996). Up until 2000, 50% of UK patients still died by age 35 years (Modell 2000), reflecting difficulties with DFO use and other issues such as the variable support patients on chelation therapy received in centres where only small numbers of patients attended. It is important to recognize that toxicity from iron overload is a long-term phenomenon, so the entire chelation history of an individual is important for outcomes, rather than simply the treatment a patient is taking when an event happens.

Table 6. Decreasing complications in cohorts of Italian patients born after DFO became available. Reproduced with permission from (Borgna-Pignatti 2004).

	BIRTH 1970-74*	BIRTH 1980-84†
Death at 20 years	5%	1%
Hypogonadism	64.5%	14.3%
Diabetes	15.5%	0.8%
Hypothyroidism	17.7%	4.9%
*IM DFO introduced in 1975 †SC DFO introduced in 1980 In 1995, 121 patients switched to DFP (censored at the time)		

Recommended treatment regimens for DFO monotherapy

Standard therapy

- **When to start DFO therapy?**
  Provided that treatment is 1) begun within 2-3 years of beginning transfusion therapy, (2) administered regularly (at least 5 times a week) and 3) administered in adequate doses, DFO has a well- established impact on survival and on cardiac and other complications of iron overload described above. In thalassaemia major, this should start before transfusions have deposited enough iron to cause tissue damage. This has not been formally determined, but current practice is to start after the first 10-20 transfusions, or when the ferritin level rises above 1,000 µg/l. If chelation therapy begins before 3 years of age, particularly careful monitoring of growth and bone development is advised, along with reduced dosage.

- **Standard dose and frequency to obtain iron balance**
  The standard aim is to balance iron input from transfusions with iron excretion through urine plus faeces. The recommended method is slow subcutaneous infusion over 8-12 hours of a 10% DFO solution, using an infusion pump a minimum of 5 days per week. In countries where pre-filled balloon infusors are available, this has been found to ease the convenience of adhering with DFO chelation. In general, average doses should not exceed 40 mg/kg until growth has ceased. The standard dose is 20-40 mg/kg for children, and up to 50-60 mg/kg for adults, as an 8-12-hour subcutaneous infusion for a minimum of 5-6 nights per week. To achieve negative iron balance in patients with average transfusion requirements, a dose of 50 mg/kg/day at least 5 days a week is required. It is important that patients with high degrees of iron loading, or those at increased risk of cardiac complications receive adequate doses, advice about compliance or consideration of alternative chelator regimens.

- **Use with vitamin C**
  Vitamin C increases iron excretion by increasing the availability of chelatable iron, but

if given in excessive doses may increase the toxicity of iron. It is recommended not to give more than 2-3 mg/kg/day as supplements, taken at the time of the DFO infusion so that liberated iron is rapidly chelated. Where a patient has just started on DFO and it has been decided to administer vitamin C, the vitamin supplement should not be given until after several weeks of treatment.

- **Dose adjustment to avoid DFO toxicity**
  At low ferritin levels, the dose of DFO needs to be reduced and DFO-related toxicities monitored particularly carefully (see below). Dose reductions can be guided using the therapeutic index (= mean daily dose (mg/kg)/SF µg/L) to keep this < 0.025 (Porter, 1989): Although a tool in protecting the patient from excess chelator, this index is not a substitute for careful clinical monitoring. Liver iron concentration has recently been advocated as a more reliable alternative to serum ferritin at low levels of body iron loading (see below).

Rescue therapy

- **Rescue to achieve negative iron balance**
  If iron has already accumulated to harmful levels (see monitoring), negative iron balance is necessary. Dose adjustment is critical to the success of chelation therapy; increased frequency, duration and dose when rescue therapy is required, and decreased dosing when body iron is well controlled. **Table 7** shows how the dose can be adjusted to achieve negative iron balance, depending on the transfusion rate. At transfusion rates > 0.5 mg/kg/day only about half of patients will be in negative iron balance at doses 35-50 mg/kg, while >50 mg/kg are required to achieve negative iron balance.

Table 7. % of responders (% in negative iron balance) by dose and transfusion rate. Adapted from (Cohen 2008).

Dose (mg/kg)	Low transfusion rate <0.3 mg/kg/day	Intermediate transfusion rate 0.3-0.5 mg/kg/day	High transfusion rate >0.5 mg/kg/day
35 - <50	76	75	52
⩾ 50	100	86	89

- **Rescue to remove cardiac iron**
  For patients with mild to moderate myocardial iron (T2* 10-20 ms), increasing the mean daily dose to 50-60 mg/kg/ day may be sufficient to improve the T2* provided that adherence to therapy can be achieved. For patients with cardiac iron of 6-10 ms, other chelation regimes have been shown to be effective, such as combination of DFP with DFO or DFX monotherapy (see below). For severe cases of cardiac iron (T2* <6 ms), other regimes need to be considered (see below). For patients with abnormal LVEF, emergency therapy is recommended.

- **Intensive therapy for other reasons**
  Prior to pregnancy or bone marrow transplantation, when avoidance of high levels of iron overload is desirable (see **Chapters 9 and 12**), intensification of therapy may be

helpful to minimize the levels of iron overload. The optimal regime has not been studied systematically but may include dose adjustment as described above with attention to adherence through goal setting.

Emergency therapy

In high risk cases with decreased LVEF, continuous infusion is potentially more beneficial than periodic infusions because it reduces the exposure to toxic free iron (NTBI), which returns to pre-treatment levels within minutes of stopping a continuous intravenous infusion (Porter 1996). The route of administration is not critical, provided that as close to 24-hour exposure to chelation as possible is achieved. Intensification of treatment through continuous, 24-hour intravenous administration of DFO via an implanted intravenous delivery system (e.g. Port-a-cath) (Davis 2000), or subcutaneously (Davis 2004) has been shown to normalise heart function, reverse heart failure, improve myocardial T2* (Porter 2013b, Anderson 2004) and lead to long-term survival, provided treatment is maintained. Some studies have included cases where for operational reasons, intensification was undertaken without continuous infusion. Continuous infusion is usually given through an indwelling line for long-term management. For emergency management before a central line can be inserted, DFO can be given through a peripheral vein, provided it is diluted in at least 100 mls of saline to avoid damage to the veins where the drug is infused. A dose of at least 50 mg/kg/day and not exceeding 60 mg/kg/day is recommended as a 24-hour infusion (Davis 2004, Davis 2000). Higher doses have been used by some clinicians, however, DFO is not licensed at these doses and the risk of retinopathy increases. Addition of vitamin C is recommended only when acute heart dysfunction has settled, which usually occurs by three months of continuous treatment (Anderson 2004). As ferritin falls, the dose but preferably not the duration of treatment can be reduced - in line with the therapeutic index (see above).

The question of whether to add DFP to intensified DFO needs to be considered. This is partly because DFP at high doses (90-100 mg/kg) was found to increase the T2* more than conventional s.c. DFO 5 days a week (Pennell 2006b) and because combined DFP + DFO has also been found to improve T2* more rapidly than conventional doses of DFO (Tanner 2007). However, these patients have baseline LVEF in the normal range and were not in heart failure but showed greater LVEF increases with the DFP containing regimes. Furthermore, these studies compared conventional intermittent non-intensified DFO with the DFP containing regimes; such low DFO doses should not be recommended for patients in heart failure. The only randomised study to examine the effect of additional DFP to intensified DFO found no difference between the two study arms with or without DFP, either with respect to LVEF or to improvements in T2* (Porter 2013b). Nevertheless, this study also showed no major extra toxicities in the study arm containing DFP, so the addition of DFP to intensified DFO would seem a reasonable course of action for patients in heart failure, provided that patient can tolerate oral administration of DFP.

Deferiprone monotherapy (Ferriprox®, Kelfer®, GPO-L-ONE®; DFP)

Deferiprone (DFP) is an orally absorbed bidentate iron chelator that began clinical trials in the UK in the 1980s. DFP was licensed in several countries from the 1990s and more recently in the US (October 2011) (Traynor 2011) for the treatment of iron overload in TM patients. The indication for treatment differs slightly in different countries (see below):

Effects on ferritin

Randomised trials comparing the effects of DFP on serum ferritin at baseline and at follow-up have been reported from the 1990s (Pennell 2006b, Ha 2006, Gomber 2004, Maggio 2002, Olivieri 1997). Pooled analysis shows a statistically significant decrease in serum ferritin at six months in favour of DFO, with no difference between the two drugs at 12 months (Pennell 2006b, Gomber 2004). There are numerous non-randomised cohort studies demonstrating a lowering of serum ferritin at doses of 75 mg/kg/day administered in three doses. The effect on SF at this dose appears greater at higher baseline ferritin values. In these studies significant decreases in serum ferritin are seen in patients with baseline values above 2,500 µg/L (Viprakasit 2013, Olivieri 1995, Al-Refaie 1992, Agarwal 1992) but not with values below 2,500 µg/L (Cohen 2000, Hoffbrand 1998, Olivieri 1995). In a recent study from Thailand, only 45% of paediatric thalassaemia patients (age > 2 years) had significant reduction of serum ferritin after 1 year at doses of over 79 mg/kg/day (Viprakasit 2013). In this study, baseline SF was the major factor that predicted clinical efficacy; patients with baseline SF>3,500 µg /L had the most significant fall of SF at 1 year. The FDA licensing agreement in 2011 concluded that "data from a total of 236 patients were analyzed, of the 224 patients with thalassemia who received DFP monotherapy and were eligible for serum ferritin analysis...the endpoint of at least a 20% reduction in serum ferritin was met in 50% (of 236 subjects), with a 95% confidence interval of 43% to 57%".

Effects on liver iron

Change in LIC from baseline after various periods of treatment with DFP has been compared with DFO in randomised studies (El-Beshlawy 2008, Ha 2006, Pennell 2006a, Maggio 2002, Olivieri 1998) and also with combination of DFP plus DFO (Aydinok 2007). One study showed initial LIC decreases at 1 year but increases in LIC at 33 months of 5 mg/g dry wt with DFP (n=18) and 1 mg/g dry wt with DFO (n=18) (Olivieri 1998). In another study an average decrease in LIC at 30 months was reported with both DFP (n=21) and DFO (n=15) (Maggio 2002). A greater 1 year decrease in LIC with DFO than DFP monotherapy was reported in several studies (Aydinok 2007, Pennell 2006a). A decrease of 0.93 mg/g dry wt with DFP (n=27) and 1.54 mg/g dry wt with DFO (n=30) (Pennell 2006) was observed at 1 year. Another study reported initial decreases in LIC at six months with both DFP and DFO, but LIC had increased by the end of the trial (Ha 2006), consistent with earlier observations (Olivieri 1998). In a randomised 1 year comparison of DFP with DFP + DFO, there was no decrease in LIC with DFP monotherapy but a decrease with combination therapy or in the DFO comparison group (Aydinok 2007). In a non-randomised prospective study using DFP, LIC increased with DFP by 28% at two years and by 68% at three years (Fisher 2003). In a recent study of paediatric patients, decrease in LIC was seen in those patients who showed a clinical response by reduction of serum ferritin and in those with a higher baseline LIC (Viprakasit 2013). In observational studies where only single biopsies were performed after several years of DFP treatment, LIC was found to be above 15 mg/g dry wt in variable proportions of patients, ranging between 11% (Del Vecchio 2002), 18% (Tondury 1998) and 58% (Hoffbrand 1998). Overall negative iron balance (decrease in LIC) with standard transfusion rates using DFP monotherapy is achieved in only about 1/3 of patients receiving 75 mg/kg (Fischer 1998).

Effects on myocardial iron

The effect of DFP monotherapy on myocardial iron has been reported in randomised studies. One compared high dose DFP (92 mg/kg/day) with s.c. DFO 5-7 days a week in patients with mild to moderate myocardial iron (mT2* 8-20 ms). The actual dose prescribed for DFO was 43 mg/kg for 5.7 days/week (or a mean daily dose of 35 mg/kg/day). The increase in mT2*

from 13 ms to 16.5 ms in the DFP group was greater than that seen in the DFO group, where an increase from 13.3 to 14.4 ms at 1 year was observed (Pennell 2006b). In another 1 year randomised study of DFP and DFO, no change in heart iron estimated by MRI (signal intensity ratio) was reported for either drug. Lower doses of DFP (75 mg/kg/day) were used in this study (Maggio 2002). In a retrospective study, higher mT2* values were seen using a multislice technique and with higher global systolic ventricular function, in patients with DFP monotherapy (n=42) than those with DFO (n=89) or DFX (n=24) monotherapies, although the mean values were in the normal range in each monotherapy category (Pepe 2011).

Effects on heart function

The effects of DFP on heart function have been documented in patients with normal baseline function (Pennell 2006b, Maggio 2002) but not in patients with baseline LVEF below the normal reference range. In a one-year randomised study of patients with normal LVEF, DFP given at high doses (92 mg/kg) increased LVEF (Pennell 2006b). In another 1 year randomised study, no difference in LVEF or other measures of LV function were seen with either DFP at 75 mg/kg/day or DFO (Maggio 2002). In a three year retrospective reanalysis of patients in the one year prospective study, follow up data showed that DFP monotherapy was associated with significant increase in LVEF in patients with LVEF in the normal range at baseline (Maggio 2012). Another retrospective study of 168 patients with thalassaemia major and baseline mean LVEF within the normal range were followed for at least 5 years while receiving monotherapy with DFO or DFP. LVEF increased in both groups but was higher in the DFP group at 3 years. However the subgroup of patients with LVEF <55% at baseline was greater in the DFO than in the DFP group (Filosa 2013).

Compliance with DFP

One study comparing compliance with DFP and DFO found rates of 95% and 72% respectively (Olivieri, 1990), while another reported rates of 94% and 93%, respectively (Pennell 2006b). The similar rate of compliance with DFP has been observed in other populations (Viprakasit 2013). As with other oral chelators, two important points should be taken into consideration: (i) compliance with any treatment tends to be higher in in the context of clinical studies than in routine use, and (ii) although compliance with oral treatment is expected to be better, the importance of constant supervision and patient support as provided when administering DFO, should not be overlooked.

Evidence of long term benefits of DFP monotherapy

Several retrospective studies have reported a survival advantage of DFP either alone (Borgna-Pignatti 2006) or with DFO (Telfer 2006) (see below), compared with DFO alone. For example, in a retrospective cohort analysis of patients treated with DFP or DFO, no deaths were reported (n=157) in the DFP arm (n=157), in contrast to 10 in DFO-treated patients (Borgna-Pignatti 2006). Other retrospective or observational studies have drawn inferences about potential advantages of DFP over DFO based on surrogate markers for survival, such as SF, myocardial T2* or LVEF (though not liver iron) (Filosa 2013, Maggio 2012, Pepe 2011). However, two systematic analyses have not found clear evidence of survival advantages of any particular chelator regime (Fisher 2013b, Maggio 2011). The Cochrane systematic review concluded: ''earlier trials measuring the cardiac iron load indirectly by measurement of the magnetic resonance imaging T2* signal had suggested DFP may reduce cardiac iron more quickly than DFO. However, meta-analysis of two trials showed a significantly lower left ventricular ejection fraction (at baseline) in patients who received DFO alone compared with those who received combination therapy using DFO with DFP'' (Fisher 2013b). Another

systematic study concluded ''There is no evidence from randomised clinical trials of different chelators or regimes to suggest that any has a greater reduction of clinically significant end organ damage, although in two trials, combination therapy with DFP and DFO showed a greater improvement in left ventricular ejection fraction than DFO used alone'' (see below). Thus while retrospective analyses encourage the view of a survival advantage with DFP monotherapy compared with DFO, this has not been confirmed by in systematic meta-analysis.

Unwanted effects with DFP

The unwanted effects of DFP and their monitoring and management are described in Appendix 2.

Recommended treatment regimens with DFP

According to the FDA, Ferriprox ® ''is indicated for the treatment of patients with transfusional iron overload due to thalassemia syndromes when current chelation therapy is inadequate'' (FDA 2011). FDA approval is 'based on a reduction in serum ferritin levels'. The European licensing Agency (EMEA) states 'Ferriprox is indicated for the treatment of iron overload in patients with thalassaemia major when DFO therapy is contraindicated or inadequate'. In Thailand and many Asian countries, DFP was registered for similar indications and is licensed for use from the age of 6.

Standard dosing and frequency

The daily dose of DFP that has been evaluated most thoroughly is 75 mg/kg/day, given in three doses. In the EU, the drug is licensed for doses up to 100 mg/kg/day but formal safety studies of this dose are limited. The standard dose of 75 mg/kg/day administered in three separate doses is therefore recommended. The drug's labeling includes charts stating how many tablets and half tablets to use per dose for patient weights ranging from 20 to 90 kg. Each 500 mg tablet is scored to facilitate tablet splitting. An oral solution is also available for paediatric use.

Dose escalation with DFP

Adjustments may be made on the basis of the patient's response but should never exceed 33 mg three times daily. Doses of 100 mg/kg/day have been given in at least one prospective study (Pennell, 2006), with no increase in reported side-effects. The relation of dose to iron balance or serum ferritin has not been reported in a single study. High dose monotherapy with DFP has not yet been prospectively evaluated for safety and effectiveness for patients with abnormal heart function, and thus combination therapy with DFP and DFO (see below) or intensive therapy with DFO as a 24-hour infusion should be recommended for this group of patients.

Age of commencement

There is less experience on the safety and efficacy of DFP in children under 6 years of age than in adults. A recent open label prospective study examined efficacy and tolerability in 73 pediatric patients, age range 3-19 years (Viprakasit 2013), as well as a similar study involving 100 children of 1-10 years old who received the liquid formulation of DFP found no specific tolerability issues that have not been previously reported in adults.

Use of vitamin C

The effect of vitamin C on iron excretion with DFP is not clear and is thus not recommended.

Safety monitoring, precautions and interactions
These are summarised in **Appendix 1** and described in **Appendix 2**.

Deferasirox (Exjade®, Asunra®; DFX)

Deferasirox (DFX) was developed as a once-daily oral monotherapy for the treatment of transfusional iron overload. The drug has been licensed as first-line monotherapy for thalassaemia major in over 100 countries worldwide, although the earliest age at which deferasirox qualifies as first-line treatment differs somewhat between the FDA and the EMEA (see Appendix 1).

Chemistry and Pharmacology
Deferasirox is an orally absorbed tridentate iron chelator, with two molecules binding each iron atom. The chemical properties and pharmacology are summarized in Table 5. The tablet is dispersed (not dissolved) in water or apple juice using a non-metallic stirrer and consumed as a drink once daily, preferably before a meal. The drug is rapidly absorbed, reaching peak concentrations of 80μM at 20 mg/kg and the long half-life of this iron-free drug allows trough concentrations of about 20 μM, providing 24hr protection from plasma labile iron (Nisbet-Brown 2003, Galanello 2003), with about 90% in the free drug form and 10% as iron complexes (Waldmeier 2010). The lipid solubility allows entry into cells, including cardiomyocytes. The majority of the drug is excreted in faeces, and metabolism is mainly to an acyly-glucuronide that retains its ability to bind iron (Waldmeier 2010). Metabolic iron balance studies show iron to be excreted almost entirely in the faeces, with less than 0.1% of the drug eliminated in urine (Nisbet-Brown 2003). The main pathway of DFX metabolism is via glucuronidation to the acyl glucuronide and the 2-O-glucuronide metabolites. Oxidative metabolism by cytochrome 450 enzymes is minor (10% of the dose) (Waldmeier 2010). The efficiency of chelation is 28% over a wide range of doses and levels of iron loading.

Evidence of effectiveness of DFX

Dose effect on serum ferritin
A dose-dependent effect on serum ferritin has been observed in several studies (Porter 2008, Cappellini 2006, Piga 2006). A prospective randomised study comparing the effects of DFX in 296 thalassaemia major patients with DFO in 290 patients, found that 20 mg/kg daily stabilized serum ferritin close to 2,000μg/L and at 30 mg/kg, serum ferritin was reduced with an average fall of 1,249 μg/L over one year (Cappellini 2006). Longer-term analysis of ferritin trends show that the proportion of patients with ferritin values <1,000 μg/L and less than 2,500 μg/L is decreasing progressively with time. At 4-5 years follow up in 371 patients, median SF had fallen to < 1500 μg/L (Cappellini 2011) and the increase in mean dose from an initial value of < 20 mg/kg to 25 mg/kg was associated with a significant fall in serum ferritin. Overall, 73% of patients attained serum ferritin levels ⩽2500 μg/L and 41% of patients achieved serum ferritin levels of ⩽1000 μg/L, compared with 64% and 12% at baseline respectively. A large-scale prospective study (EPIC) has examined the interaction between dose and SF response in large scale studies involving 1,744 transfusion-dependent anaemias, including 1,115 with TM (Cappellini 2010). The initial dose of deferasirox was 20 mg/kg/day for patients receiving 2-4 packed red blood cell units/month, and 10 or 30 mg/kg/day for patients receiving less or more frequent transfusions, respectively. Dose adjustment were made on the basis of ferritin trends at 3 monthly intervals. A significant though modest overall fall in ferritin was seen at 1 year. In a recent substudy, the largest SF decrease of

-1,496 µg/L/year was noted in patients with the highest baseline SF values (baseline median SF 6,230 µg/L) (Porter 2013a). These patients were treated with DFX at high dosage (35-40 mg/kg/day), which are therefore doses now recommended for heavily iron overloaded patients.

Dose effect on liver iron and iron balance

Metabolic balance studies showed that excretion averaged 0.13, 0.34 and 0.56 mg/kg/d at DFX doses of 10, 20 and 40 mg/kg/d respectively, predicting equilibrium or negative iron balance at daily doses of 20 mg and above (Nisbet-Brown 2003). In a longer term randomised prospective study in 586 thalassaemia patients aged 2 to 53 years (with half of patients <16 years old), iron balance with DFX (n=290) assessed by serial LIC determination was achieved at 20 mg/kg/day, with mean LIC remaining stable over one year (Cappellini 2006). Negative iron balance was achieved at 30 mg/kg/day, with a mean LIC decrease of 8.9 mg/g dry wt (equivalent to a decrease in body iron of 94 mg/kg body weight) over one year. These are average trends and a closer analysis shows that the blood transfusion rate influences the response to treatment (Cohen 2008) (Table 8). This shows that negative iron balance over 1 year (response rate) is increased as doses increase, and that the response rate is less at high transfusion rates, who therefore required higher doses.

Table 8. % of responders (% in negative iron balance) by dose and transfusion rate. Adapted from (Cohen 2008).

Dose (mg/kg)	Low transfusion rate <0.3 mg/kg/day	Intermediate transfusion rate 0.3-0.5 mg/kg/day	High transfusion rate >0.5 mg/kg/day
10	29	14	0
20	76	55	47
30	96	83	82

At 4-5 years of follow up, the percentage of patients with LIC values <7 mg/g dry wt by biopsy increased from 22% at baseline to 44% (Cappellini 2011). A more moderate reduction in LIC occurred in children under six years old, despite the administration of an average dose of 21.9 mg/kg in this subgroup. However, these patients had the highest mean transfusional iron intake. In a recent liver MRI analysis of 374 patients enrolled on the EPIC study (Porter 2013a), response to DFX was analyzed according to baseline levels of iron overload. In patients with a high baseline LIC of 27.5 mg/g dry wt, LIC decreased by 6.9 mg/g dry wt at one year at doses of 25-35 mg/kg/day. In patients with LIC of 32 mg/g dry wt the decrease was 7.3 mg/g dry wt and 35-40 mg/kg/day, respectively. Thus, provided adequate doses are given, there is a good response to DFX across the full range of baseline LIC values (Porter 2013a).

Iron balance and safety in children

DFX was the first chelator to be formally assessed in children as young as 2 years old. Approximately 50% of patients in 5 clinical studies that included 703 patients were children

aged <16 years. The drug appears to be tolerated in children as well as in adults. Importantly, no adverse effects on growth or skeletal development were observed at a dose of 10 or 20 mg/kg /day (Piga 1988). In another observational study of chelation-naive transfusion-dependent children (aged < 5 years) with SF > 1000 µg/L at baseline, DFX or DFO was prescribed to maintain serum ferritin levels between 500 and 1000 µg/L. With a median follow up of 2.3 years for DFX (n = 71) and 2.8 years for DFO (n = 40), DFX was shown to be well tolerated and at least as effective as DFO in maintaining safe serum ferritin levels and normal growth progression (Aydinok 2012b).

Effect on myocardial T2*

Improvement in mT2* was first reported in a retrospective analysis of effects on myocardial T2* after 1 and 2 years (Porter 2010, Porter 2005a). Prospective data demonstrated the efficacy of DFX in improving myocardial T2*over a range of mT2* from 5-20 ms, with 41% having severe myocardial iron loading <10 ms at baseline (Pennell 2010). In a prospective trial, 114 patients with high mean baseline LIC (mean 28 mg/g dry wt) were treated with DFX for up to 3 years (Pennell 2012), receiving mean actual doses of 33, 35, and 34 mg/kg/day during the 1st, 2nd and 3rd years, respectively. Higher mean doses of 37 mg/kg per day were received by patients with baseline T2* between 5 and <10 ms, compared with those between 10 and 20 ms (32 mg/kg per day). Of the 114 patients initially enrolled, 101 continued into the 2nd year, 86 completed two years of treatment and 71 entered into a third year. There was year by year significant improvements in mT2*; from 12.0 ms at baseline to 17.1 ms at 3 yrs, corresponding to a decrease in cardiac iron concentration (from 2.43 mg/g dry wt at baseline, to 1.80 mg/g dry wt. After three years, 68 % of patients with baseline T2* between 10 and <20 ms benefited from normalization of T2*, and 50% of patients with baseline T2*>5 to <10 ms at baseline improved to 10 to <20 ms. There was no significant variation in left ventricular ejection fraction over the three years and no deaths occurred. Tolerability was similar to other DFX studies in TM over the doses up to 40 mg/kg/day.

In a 1 year randomised prospective study (CORDELIA) 197 patients with T2* of 6-20 ms and no signs of cardiac dysfunction were randomised to DFX (target dose 40 mg/kg/day) or subcutaneous DFO treatment (50-60 mg/kg/day for 5-7 days/week) (Pennell 2014). Baseline LIC was high in both DFX (mean 29.8 mg/g dry wt) and DFO treated patients (30.3 mg/g dry wt), with 73% of patients having baseline LIC >15 mg/g dry wt. The geometric mean (Gmean) myocardial T2* improved with DFX from 11.2 ms at baseline to 12.6 ms at 1 year (Gmeans ratio 1.12) and with DFO (11.6 ms to 12.3 ms, Gmeans ratio 1.07). This study established non-inferiority of DFX vs. DFO for cardiac iron removal in this patient population. LVEF remained stable in both arms and the frequency of drug-related adverse events was comparable between DFX (35.4%) and DFO (30.8%). Taken together, these studies show that DFX is an effective treatment for patients with increased heart iron with mT2* >5-20 ms. It also demonstrates response in patients with high levels of baseline mT2* (5-10 ms), as well as those with high levels of baseline LIC or SF. As with other chelation regimes, high levels of baseline heart iron (<10 ms) will typically take several years to clear, but the risk of developing heart failure during this time appears very low (see below), provided treatment is monitored.

Effects on heart function

In the above studies, even though mT2* values at baseline were as low at 5-6 ms and the proportion of patients with mT2* <10 ms was significant (17.2-33%), LVEF remained stable, and there were neither deaths nor episodes of symptomatic heart failure observed. Only

one case of atrial fibrillation and one case of cardiomyopathy were reported. According to risk analysis of heart failure in TM from other cohorts, the risk of developing cardiac failure was expected to be substantial, with a relative risk 160 fold higher for patients with T2* < 10 ms (Kirk 2009a). The stability of LVEF and the absence of heart failure in this otherwise high risk group of patients suggests that DFX renders effective prophylaxis for heart failure, even in patients with T2* values of 5-10 ms. This may be related to the 24hour 'protection time' against labile iron that results from the long plasma half-life of DFX (Daar 2009). Deferasirox has not been evaluated in formal trials for patients with symptomatic heart failure or LVEF < 56%, therefore at this time other chelation options are recommended for such patients.

Evidence of long term survival benefits of DFX

More than 5,900 patients have been enrolled in prospective trials but these, with some exceptions, have typically been designed for short term evaluation. Up to 5 years of follow up have now been reported in one prospective clinical trial from the initial registration studies, which provides useful information about risk and benefit with this treatment (Cappellini 2011). Other prospective data of patients with myocardial T2* of 5-20 ms and high levels of liver iron but without complications, provides further insight into comorbidities and mortality in high-risk subjects. The stability of left ventricular function, lack of progression to heart failure and absence of any deaths are notable features of the prospective 3-year EPIC and 1-2 year CORDELIA cardiac studies, despite including patients at high risk of cardiac decompensation, with mT2* levels as low as 5 ms (Pennell 2012) or 6 ms (Pennell 2014).

Convenience and impact on quality of life

Convenience and quality of life on DFX, as with other oral chelation regimes, are expected to impact on adherence and hence survival. This is likely to have a greater impact outside formal clinical studies, where adherence is generally better than in routine clinical use. Studies comparing satisfaction and convenience of DFX with DFO in thalassaemia major show a significant and sustained preference for DFX (Cappellini 2007). In a randomised comparison, total withdrawals in DFX-treated patients was 6% at one year, compared with 4% with DFO (Cappellini 2006). This compares with a dropout rate of 15% at one year with DFP, although these are not matched populations (Cohen 2000). In the large scale EPIC study, patients reported improved quality of life (estimated by SF36 scores) and greater adherence to chelation therapy compared with baseline before starting DFX (Porter 2012).

Recommended treatment regimens with DFX

Recommended standard dosing

Deferasirox is taken orally as a suspension in water once daily, and preferably before a meal. A starting dose of 20 mg/kg is recommended for thalassaemia major patients who have received 10-20 transfusion episodes and currently receive standard transfusion at rates of 0.3-0.5 mg of iron/kg/day. In those patients in whom there is a higher rate of iron intake from transfusion (>0.5 mg/kg/day), or in patients with pre-existing high levels of iron loading where a decrease in iron loading is clinically desirable, 30 mg/kg/day is recommended. For patients with a low rate of iron loading (<0.3 mg/kg/day), a dose of 10-15 mg/kg may be sufficient to control iron loading.

Age of commencement

The labeling for age of commencement differs in countries that follow US licensing from those that adhere to EU licensing (**Appendix 1**). However, based on prospectively randomised

studies of DFX in children as young as two years of age, some recommendations can be made. A fall in LIC has been seen across all age groups analysed, with no age-related adverse effects. In particular, no adverse effects on growth, sexual development or bones have been observed (Piga 2006). Deferasirox also appears to be palatable to children at this young age. On the basis of present knowledge, the criteria for starting treatment (ferritin level, age, number of transfusions) are similar to those of DFO. However, a target of 500-1000 µg/L appears to be achievable with DFX without additional toxicity issues, provided that doses are adjusted downwards as SF values fall towards 500 µg/L.

Rescue therapy to achieve negative iron balance

When body iron has accumulated to high levels (see monitoring), negative iron balance is required. The proportion of patients in negative iron balance at a given dose is partially dependent on the rate of iron loading (see above). Doses of up to 40 mg/kg/day are recommended for patients with LIC or SF values and are now licensed at this dose (Porter 2013a). Dividing the dose as a twice daily dose has been used in some patients who fail to achieve negative iron balance, despite these higher doses (Pongtanakul 2013). Some patients have taken DFX after rather than before food, with apparently improved efficacy. This is consistent with the known effects of food on GI absorption (Galanello 2008).

Rescue therapy for patients with mild to moderate myocardial iron (5-20 ms)

On the basis of prospective studies these patients can be successfully treated with DFX, resulting in preservation and stabilisation of LV function. Doses of up to 40 mg/kg have been used and are advisable in patients with very high levels of liver iron or serum ferritin.

Rescue therapy for patients with severe myocardial iron (< 6 ms)

Prospective clinical trials with DFX monotherapy have been confined to patients with mT2* values ≥6 ms. For patients with mT2* <6 ms, other alternative chelation regimes are recommended.

Emergency therapy for patients with reduced LVEF or symptomatic heart failure

DFX has not been formally evaluated in prospective trials for such patients and is therefore not recommended.

Other indications and contraindications

DFX is contraindicated in patients with renal failure or significant renal dysfunction (see below). Caution is recommended for patients with advanced liver disease and hepatic decompensation.

Unwanted effects with DFX

Unwanted effects of DFX and their monitoring and management are described in **Appendix 2**.

Combination therapies

Concept and pharmacology of combination therapies

The term 'combination therapy' has been used to cover a variety of approaches to improve outcomes if monotherapy proves inadequate. In principle, two chelators can be given at the same time (simultaneously), or one after the other (sequentially). True combination, where two chelators are present in the blood at the same time, has been used relatively rarely

compared with sequential regimes. Some investigators have used the term 'alternating therapy' to describe the use of two drugs administered on alternate days, reserving the term 'sequential therapy' for when DFO is given at night and DFP during the day. In practice, regimes may involve both a component of 'sequential' and 'alternating' therapy such as when DFO is given three times a week (alternate nights) and DFP every day. Most commonly used regimes have tended to give DFP daily at standard doses, combined with varying frequency and dosing of DFO. More recently, combinations of DFX with DFO, or DFX with DFP have been evaluated. In this instances, both drugs may be present in plasma or intracellularly for at least part of the time owing to the half-life of DFX and its extended time in the plasma - for up to 24hr.

The pharmacology and mechanisms of action in combining chelators is dependent on whether the drugs are present in cells or plasma at the same time. By giving DFO at night and DFP by day, 24-hrs of exposure to iron chelation can be achieved (similar exposure to that achieved with 24-hrs desferrioxamine infusion, or once daily deferasirox). This has the theoretical advantage of 24hr protection from labile (redox active) iron (Cabantchik 2005). If the drugs are given at the same time (simultaneously), they may interact in a process that involves the 'shuttling' of iron, which may lead to additional chelation of iron from cells or plasma NTBI (Evans 2010) and so improved efficiency of iron chelation. On the other hand, there is also the possibility of chelation from metalloenzymes, leading to increased drug-related toxicity, but this has not been an issue clinically. The use of DFX, which is present in plasma 24hrs/day, together with DFO by intermittent infusion provides 24hr chelation, with decreases in LPI and NTBI (Lal 2013). Simultaneous exposure to two chelators may also result in synergistic removal of cellular iron. This has been demonstrated in cell culture with combinations of all three chelators (Vlachodimitropoulou 2013).

Combined DFO and DFP

Combinations of these chelators has been studied more extensively than other chelator combinations so far. A variety of regimens involving combinations of DFP and DFO have been used, either in the context of a formal trial or on an ad hoc basis, usually when monotherapy with DFO or DFP has failed to control iron overload or its effects. These have been detailed elsewhere (Porter and Hershko 2012). Here some of the key studies providing useful evidence are described.

Evidence of efficacy of combined regimes

- **Effects of sequential use on serum ferritin**
  One study (Mourad 2003) found the decrease in SF achieved using five days of DFO monotherapy (n=11) to be similar to that achieved with two nights of DFO plus seven days of DFP at 75 mg/kg (n=14). Another randomised study involving 30 patients and three different treatments (Gomber 2004) found that the decrease in SF was greatest with five nights of DFO, albeit not significantly different from that achieved with a combined treatment of DFO two nights a week, plus DFP seven days a week. A further randomised study involving 60 patients receiving 'alternating' therapy (Galanello 2006) found no difference in SF in patients randomised to combined treatment (two days DFO at 33 mg/kg + seven days DFP at 75 mg/kg), or to DFO five nights a week at 33 mg/kg. In another randomised study from Turkey (Aydinok 2007), SF decreased more with combination therapy than DFP monotherapy but similarly to DFO monotherapy. In a randomized study of 65 patients (Tanner 2007), serum ferritin was decreased more by

combined treatment (DFO five days a week plus DFP seven days a week) than with standard DFO monotherapy (40 mg/kg five times a week). A 5-year follow up randomized clinical trial (Maggio 2009) also showed a greater SF reduction with sequential DFP (75 mg/kg/day-4 days/week)-DFO (50 mg/kg/day-3days/week) compared with DFP alone on reducing serum ferritin, with comparable adverse effects and cost. However, survival trend of both groups was not significantly different. Taken together, these studies show that serum ferritin can be controlled with a relatively low frequency of DFO given twice a week when combined with DFP at standard doses (75 mg/kg/day).

- **Effects of sequential use on liver iron**
  When an alternating DFP + DFO regime was compared to DFO monotherapy, a baseline LIC of <7 mg/g dry wt was maintained on average in both arms of the study (Galanello 2006). A different prospective randomised study found LIC reduction in either DFO monotherapy or DFP + DFO (2 times weekly) combination therapy, but not with DFP monotherapy at 75 mg/kg/day (Aydinok 2007). In another study comparing LIC changes using DFP + DFO or DFO monotherapy (5 times a week), a greater improvement in liver T2* (as a surrogate measure of LIC) was seen with combination therapy than with DFO alone (Tanner 2007).

  In another randomised study in patients with heart failure who received DFO with or without DFP, there was decrease in LIC and ferritin in the combination arm but not with monotherapy (Porter 2013b). These studies did not give definitive results with respect to the comparative LIC effects of combination therapy relative to other therapies, although they do support combination therapy being more effective than DFP monotherapy. The relative efficacy of combination compared with DFO monotherapy most likely depends on the dosing and frequency of DFO used in the different regimes.

- **Effects on heart function and T2***
  In a randomised controlled study of 65 patients without heart failure but with moderate heart iron loading (T2* 8-20 ms) and LVEF in the normal reference range, changes in myocardial T2* with combined DFP at 75 mg/kg seven days a week plus DFO five days a week were compared with patients on standard DFO treatment five times a week (Tanner 2007). The study showed that LVEF increased within the normal range by approximately 2.5% in the combination arm and 0.5% in the DFO monotherapy arm. Myocardial T2* improvements were seen with both treatments, but were greater in the combination arm. For high risk patients with decreased LVEF or with symptomatic heart disease, a prospective randomised study showed significant improvement in LVEF and T2* in patients receiving either DFO intensification or DFO intensification plus DFP (Porter 2013b). No statistical difference between the two study arms was found with respect to these variables, although larger study numbers would be required to show a 5% difference in LVEF response. Observational studies have also reported changes in heart function with combined treatment. In 79 patients with SF>3000 µg/L treated with a variable DFO regimen plus DFP at 75 mg/kg seven days a week for 12 to 57 months, there was an increase in LVEF by echocardiography (Origa 2005). In an observational study of 42 patients with sequential use of treatment over three to four years (DFP 75 mg/kg/day plus DFO two to six days a week), the LV shortening fraction improved (Kattamis 2006). In another observational multicentre study, changes in mT2* and LVEF were examined in patients who were receiving combined DFO + DFP (N=51), DFP (N=39) or DFO (N=74) monotherapy (Pepe 2013). The proportion of patients that maintained a

normal heart T2* value was comparable between DFP and DFO when compared with both monotherapy groups. Increase in mT2* was greater in patients on DFO + DFP, or DFP than with DFO monotherapy, but did not differ between DFO + DFP and DFP, with combination therapy not showing an additional effect on heart function over DFP alone. Monotherapy with DFX is usually effective at improving T2* across a full range of LIC levels (Pennell 2012). The effects of DFX on T2* has not been compared directly with DFP either alone, or in combination with DFO. If the imperative is to have improvement as rapidly as possible (for example in preparation for pregnancy or prior to bone marrow transplant), then high dose DFP monotherapy (>90 mg/kg) or regular doses of DFP in combination with standard DFO 5 days a week should be used, as combination of DFP and DFO may result in more rapid improvement in cardiac iron load and increases in LVEF, which has not been observed with other chelation regimes.

- **Long term effects on survival**
  Several retrospective analyses have reported a benefit in survival compared with DFO alone. In one such analysis, of 544 patients with ß-thalassemia from Cyprus treated between 1980 and 2004, 304 switched to combination chelation therapy from 1999 as part of their regular treatment. The authors reported a worsening survival in Cyprus up until 2000, followed by an improved subsequent survival which they attributed to switching to combination therapy (Telfer 2009, Telfer 2006). 75 patients came off combination therapy because of agranulocytosis (5%), recurrent neutropenia (2.9%), gastrointestinal disturbances (5.6%), arthralgia (1.6%), allergic reactions (0.7%), weight gain (0.7%), increased liver enzymes (0.3%), non-adherence to DFO (3.3%), pregnancy (2.6%), and other reasons (2%). Some authors have achieved very low serum ferritin values using a 'flexible' approach to combination therapy without any reported side effects, although the reasons for giving combination therapy were not clear (Farmaki 2010). Controlled trials demonstrating improvement in disease-related symptoms, organ function, or increased survival are, however, lacking. Meta-analysis reviewing multiple trials found no statistically significant variations in heart T2* signal during associated or sequential versus mono-therapy treatment (p=0.46 and p=0.14, respectively) (Maggio 2011). This analysis did find "improved ejection fraction during combination associated or sequential (combination) versus monotherapy treatment (p=0.01 and p=0.00001, respectively)". However "these findings do not support any specific chelation treatment. The literature shows risks of bias, and additional larger and longer trials are needed" (Maggio 2011).

- **Safety of combined DFO + DFP treatment**
  Formal safety data on combined treatment are limited. The side effects described above are largely consistent with the known effects of the individual chelating agents, with the possible exception of cerebella syndrome in a single case. Tolerability of simultaneous combinations may differ from sequential use, but this has not been formally studied.

- **Conclusions and possible treatment regimens**
  Combinations of these two drugs are useful, especially when various monotherapy regimes have failed to control either liver iron or cardiac iron. This is most commonly a consequence of pharmacological differences with DFP, or for reasons of poor adherence to DFO. In general, if a patient is not doing well with DFP monotherapy, combined treatment offers an additional option to improve iron balance. For patients not doing well with DFO monotherapy for reasons of compliance, and where dose intensification has failed, combined treatment has been used as a way of decreasing the frequency at

which DFO is needed to maintain SF and iron balance. For patients with very high levels of heart iron or cardiac dysfunction without frank heart failure, 24-hour treatment with DFO and daily therapy with DFP should be strongly considered.

Combined DFX and DFO

Experience with this combination is relatively limited compared with the above regimes. Two prospective studies have evaluated this combination. In the first, 22 patients were studied over 12 months of DFX at 20-30 mg/kg daily plus DFO at 35-50 mg/kg on 3-7 days/week. Median LIC was shown to decrease by 31% and median ferritin by 24%. All 6 subjects with elevated myocardial iron showed improvements in MRI T2*. Both NTBI and LPI fell significantly. Tolerability was consistent with that seen previously with individual treatments (Lal 2013). A larger prospective study has examined 60 patients with severe liver and heart iron overload (cardiac T2* 5-10 ms) given DFX 20-40 mg/kg/d 7 days per week, plus DFO 40 mg/kg/d 5 days per wk for ≥8 hrs/d (Aydinok 2013). Results up to 2 years show a reduction in SF of 44% and 52% in LIC, and an increase in cardiac T2* of 33% (Aydinok 2014). Improvement in mT2* were greater in patients with baseline LIC <30 than those >30 mg/g dry wt. LVEF remained stable during the study. Tolerability was consistent with that seen with monotherapy regimes.

Combined DFX and DFP

Experience with the combination of these two drugs is currently even more limited. Single case reports suggest that this is an effective regime (Voskaridou 2011). One study reported combined use in 16 patients for a period of up to 2 years with decrease of total body iron load as estimated by serum ferritin, LIC and MRI T2* indices (Farmaki 2010). The incidence of adverse events was minor compared to the associated toxicity of monotherapy of each drug. No new onset of iron overload-related complications was demonstrated, with reversal of cardiac dysfunction in 2/4 patients and significant increase in mean LVEF. More recently, preliminary results of a larger randomised trial have been presented comparing this combination with DFP monotherapy (Elalfy 2013). In 96 patients in Egypt, two combination regimes were compared over 1 year: DFP 75 mg/kg in two divided doses was given in both regimes and combined with either DFX 20 mg/kg once daily, or with overnight DFO at 40 mg/kg (the frequency of DFO is not stated in the abstract). SF, LIC, and mT2* improved significantly in both groups and no serious adverse events were reported during the study in either treatment group. The authors reported improvement in quality of life in a greater number of patients in the arm containing DFX, than those treated with DFO. These findings are encouraging but further studies are needed to clarify the tolerability of this approach, and to determine how this might be used most effectively and safely. The optimal relative doses and frequency of each drug also need to be determined, which may vary depending on the degree of cardiac or overall iron overload.

Which chelation regime, when and how much?

Standard therapy for obtaining iron balance

The licensing of individual chelators, specified in the country where the treatment is prescribed should act as an initial guide on when to start the therapy and at what dose (see Appendix 1). Standard first line doses have been discussed in detail above, and depend in part on the rate of transfusional iron loading. Starting chelation before overload has built up, or irreversible damage has occurred is critical to success. With DFO, chelation was often withheld until the SF had reached 1000 µg/L because of fears toxicity would have on growth, ears and eyes

at low levels of body iron. It may be that with new chelator regimes that chelation can be started earlier, however, information about this is limited at present. In practice, the exact timing of starting chelation is currently constrained to some extent by the licensing of the compound by regulatory authorities, which differs somewhat between countries. If a patient is failing on first line therapy, dose adjustment and attention to adherence (practical as well as psychological support) are the next steps. If this fails then regime adjustment can be considered, depending on the circumstances - some of which described below.

Iron load too high or is increasing – rescue therapy to achieve negative iron balance

If body iron load builds up because of a delayed in starting chelation therapy, inadequate dosing, and poor adherence or because of poor response to an individual monotherapy, rescue therapy is required by one or more of the following:

(a) Increasing the dose of chelation a, b, c
(b) Increasing frequency of the chelator (improving adherence d, or increasing prescription advice)
(c) Switching chelator regimen
(d) Rotating e or combining f chelators

a. DFO monotherapy is effective at producing negative iron balance if it is given in sufficient doses and sufficient frequency, but adherence is often a problem.
b. Dose escalation of DFX is effective at producing negative iron balance (see Table 7). Doses >35 mg/kg and up to 40 mg/kg are effective in patients with high LIF or SF.
c. DFP monotherapy is likely to achieve iron balance at 75 mg/kg in only about one third of patients, with average transfusion rates. DFO is often added to this.
d. If adherence is the major reason for a regime not working, every effort should be made by the health team to support the patient and their family in achieving better adherence.
e. Rotation of individual monotherapies (sequential chelation) can be helpful in managing individual patients, often for reasons of adherence as much as for specific complications.
f. True 'combination therapy' (where two chelators are combined with some degree of overlap pharmacologically) is widely practiced, although not specifically licensed. This has been shown to be useful in individual patients when monotherapy is inadequate, either to control iron balance or to control iron distribution, particularly in the heart.

Mild increase in cardiac iron (T2* 10-20 ms) - rescue therapy to remove heart iron

DFO, DFP and DFX monotherapy are all effective at decreasing heart iron, but need to be given without interruption for optimal effects and at adequate doses. The immediate risk of heart failure is low, provided that the patient remains on chelation therapy without interruption (Kirk 2009b). Regular daily monotherapy at optimal doses (often an increase from current dose or frequency) will usually improve heart iron but normalisation of heart iron can take several years of consistent therapy and sequential monitoring of T2* is required. Monotherapy with DFX is usually effective and recent work shows that DFX is effective at improving T2* across a full range on LIC concentrations (Pennell 2014, Pennell 2012). If the imperative is to do this as rapidly as possible (for example in preparation for pregnancy or prior to bone marrow transplant), then high dose DFP monotherapy (>90 mg/kg) or regular doses of DFP in combination with standard DFO 5 days a week should be used, rather than

DFO alone when given s.c. 5 days a week. DFX has not been compared directly with DFP either alone or in combination with DFO. If there is no trend of improvement in T2* with DFO, DFP or DFX monotherapy, then combined DFP and DFO should be considered.

Cardiac T2* < 10 ms - rescue therapy for heart iron

The risk of developing heart failure increases with lower cardiac T2*, especially when values drop below 10 ms (i.e. higher heart iron). However, if continuous chelation therapy is given, heart failure may be prevented even before the T2* is corrected. This was has been shown for continuous 24h DFO, with high dose DFX in a population where T2* was 6-10 ms, and in patients treated with different combination regimes (DFO+DFP, DFO+DFX). Patients with T2* ⩽ 6 ms, are a very high risk group for developing heart failure. This group has not been evaluated extensively with interventional studies (expect people with heart failure). There is some experience of treating these patients with DFO + DFP, but randomised trials did not include patients with T2* values < 8 ms (Tanner 2007). In the absence of formal comparisons with other regimes, the combination of DFO (given as often and as continuously as possible) with DFP at standard doses is recommended. DFX monotherapy at doses >30 mg/kg/day has also been shown to be effective for patients with T2* >5 ms and normal heart function. If patients also have high levels of body iron as well as heart iron, it is important that the regime also reduces total body iron.

Patients with heart failure - reverse heart failure

If chelation therapy is taken regularly clinical heart failure is now rare. Reversal of heart failure requires continuous DFO therapy and can occur within a few weeks of starting treatment. This will not succeed in all cases, but if started early in the development of heart failure, is usually effective. The addition of DFP in these circumstances may be beneficial, although a small randomised comparison did not show a difference with our without DFP (Porter 2013b). Once reversal of heart failure has been demonstrated clinically and with myocardial MRI or echocardiography, continuation of the same therapy is recommended until the cardiac T2* improves to T2* above 8 ms, depending on the starting T2* this may take as long as a year. The key to success is the timely introduction of intensification and the maintenance of intensive treatment after the heart failure has been corrected.

Downward adjustment of chelator dose if body iron falls rapidly or reaches low levels

An increasingly common problem for patients who respond well to a chelation regime is that the clinician does not recognize this and/or does not adjust the dose downward soon enough to prevent toxicity from over chelation. This is more likely in centres without long term or regular experience in monitoring and prescribing iron chelation. Regular monitoring for SF trends (1-3 times monthly) and for the known toxicities of each chelator are minimum requirements. The general principle of downward dose adjustment with rapidly falling body iron loads is clear, but the specifics as regards how much and when are less clear with some of the newer chelator regimes, and these are discussed in the respective sections on individual chelators. In general, the risk of over chelation with DFO increases when the SF is low relative to the dose. This has not been analysed systematically with other chelation regimes. With DFX, low levels of SF can be achieved even in patients not receiving transfusion, provided the doses are low (5-10 mg/kg), as SF values fall below 500µg/L (Taher 2013). Cases of toxicity from over chelation have been observed when SF are higher than this, if the rate of decrease is rapid. DFX dose adjustment consideration should be made at the first sign of increasing serum creatinine values (as a late event associated with falling SF or LIV values). With DFP it is not clear whether to or how to adjust dosing at low levels of SF or with rapid decrements in SF.

Summary and Recommendations

1) Uncontrolled transfusional iron overload increases the risks of heart failure, endocrine damage, liver cirrhosis and hepatocellular carcinoma **(B)**.
2) Liver iron concentration can be used to calculate total body iron, and serum ferritin is an approximate marker of LIC **(B)**.
3) Chelation therapy is an effective treatment modality in improving survival, decreasing the risk of heart failure and decreasing morbidities from transfusional iron overload **(A)**.
4) Chelation therapy at the correct doses and frequency can balance iron excretion with iron accumulation from transfusion **(A)**.
5) Absolute change in total body iron in response to chelation can be calculated from change in LIC **(B)**.
6) Direction of change in body iron in response to transfusion and chelation can usually but not always be estimated from the trend in serum ferritin **(B)**.
7) Prevention of iron accumulation using chelation therapy is preferable to rescue treatment because iron mediated damage is often irreversible, and removal of storage iron by chelation is slow - particularly after it has escaped the liver **(B)**.
8) Response to chelation is dependent on the dose applied and the duration of exposure **(A)**.
9) Response to chelation is affected by the rate of blood transfusion **(B)**.
10) Heart iron is accumulates later than liver iron, and is rare before the age of 8 years; affecting a subset of patients **(B)**.
11) Chelation of storage iron from the liver tends to be faster than from myocardium **(B)**.
12) Heart storage iron concentration is directly related to the risk of heart failure, which can be reliably estimated by MRI (e.g. cardiac T2*), provided the centre performing the measurement uses a validated method that has been independently calibrated **(B)**.
13) Chelation can reverse iron mediated heart dysfunction rapidly (weeks) by rapid chelation of labile iron, if 24h chelation cover is achieved **(A)**.
14) Chelation therapy removes myocardial storage iron slowly (months or years) **(A)**.
15) Over chelation increases side effects from chelation therapy, and doses should therefore be decreased as serum ferritin or liver iron levels fall (demonstrated most clearly with DFO) **(B)**.
16) The optimal chelation regime must be tailored for the individual and will vary with their current clinical situation.
17) Chelation therapy will not be effective if it is not taken regularly – a key aspect of chelation management is to work with patients to optimize adherence **(B)**.

Appendix 1

Table A1. Licensed indications, and precautions for chelation in thalassaemia.

CATEGORY	DFO (DESFERRIOXAMINE)	DFP (DEFERIPRONE)	DFX (DEFERASIROX)
Children age 2-6	First line for TM	Insufficient information for licensing	First line in USA Second line when DFO contra-indicated or inadequate in Europe
Children age > 6 and adults	First line TM	If other chelation (FDA 2011) or DFO not tolerated or ineffective	First line TM First line NTDT
Route	s.c. / i.m. or i.v injection	Oral, tablet or liquid	Oral, dispersed tablet
Dosage and frequency	20 - 60 mg/kg 5 -7 x / week, 50 mg/kg in EU Children's dose up to 40 mg/kg	75 -100 mg/kg/day in 3 divided doses daily	20-40 mg/kg/day once daily. Lower doses in NTDT
Contra-indications	- Pregnancy (but has been used in 3rd trimester) - Hypersensitivity	- Pregnancy - History of neutropenia or condition with underlying risk of cytopenia - Hypersensitivity including Henoch Schönlein purpura: urticaria and periorbital oedema with skin rash	- Pregnancy - Hypersensitivity - Estimated creatinine clearance <60 ml/min - Hepatic impairment or renal failure

CATEGORY	DFO (DESFERRIOXAMINE)	DFP (DEFERIPRONE)	DFX (DEFERASIROX)
Precautions	- Monitor ferritin: if it falls to <1000 µg/L , reduce dose (so mean daily dose/ferritin remains <0.025) - Monitor audiometry regularly, particularly as ferritin falls - Monitor eyes regularly including electroretinography if on high doses - Fever suggestive of septicemia with organisms that used ferrioxamine (yersinia, klebsiella) - Renal failure or diminishing renal function with other comorbidities	- Measure neutrophil count (ANC) before starting and monitor ANC weekly - For neutropenia : ANC < 1.5 x 10^9/L) interrupt treatment - For agranulocytosis (ANC < 0.5 x 10^9/L), consider hospitalization - Advise patients to report immediately symptoms of infection: Interrupt if fever develops - Monitor for symptoms of arthropathy - Monitor liver function regularly - No guidance on dose adjustment at low ferritin	- Monitor creatinine trends for 1st 4 weeks after starting or after dose escalation then monthly - If rapid fall in serum ferritin to <1000 µg/L- dose reduce. If ferritin 500 µg/L consider very low doses^ - Proteinuria may occur: occasionally with renal tubular acidosis. Monitor urine protein regularly - Prescribing to the elderly: non-fatal gastrointestinal bleeding, ulceration, and irritation may occur: caution with drugs of known ulcerogenic or hemorrhagic potential, (e.g. NSAIDs, corticosteroids, oral bisphosphonates, and anticoagulants) - Hypersensitivity reactions - Monitor liver function regularly

CATEGORY	DFO (DESFERRIOXAMINE)	DFP (DEFERIPRONE)	DFX (DEFERASIROX)
Potential drug interactions	- Co-administration with prochlorperazine: may lead to temporary impairment of consciousness. - Gallium-67: Imaging results may be distorted by rapid urinary excretion of Desferal bound gallium-67. Discontinuation 48 hours prior to scintigraphy advisable	- Theoretical interactions with UGT1A6 inhibitors (e.g. diclofenac, probenecid, or silymarin (milk thistle) - Avoid concomitant use with drugs associated with neutropenia - Gallium-67 as with DFO - Oral preparations containing polyvalent cations (e.g., aluminum containing antacids, and zinc) allow at least a 4-hour interval	- Theoretical interactions with drugs metabolized by CYP3A4 e.g. midazolam - Theoretical interactions with drugs metabolized by CYP1A2: e.g. Theophylline - Gallium-67 as with DFO - Oral preparations containing polyvalent cations as with DFP

^ Drug labelling recommends stopping when ferritin 500 µg/L but this risks rebound labile iron and see-saw pattern of iron overload. Consider gradual dose reduction as ferritin falls <1000µg/L.

Appendix 2

Unwanted Effects of Iron Chelators; Monitoring and Management

Unwanted effects of chelation therapy are generally more likely at high chelator doses and at low levels of iron overload, and possibly in association with high rates of reduction in body iron. There is more information about the relationship of these variables with DFO than with DFX, and little information is available about the effect of DFP dosing on unwanted effects. Although the licensing of each chelator includes some recommendations about how to monitor for unwanted effects, in this this appendix we have placed these in the context of overall management of TM patients.

Evidence for the relative frequency of adverse events in randomised studies have been aggregated from 18 trials by systematic review (Fisher 2013a, Fisher 2013b). This concluded "Adverse events are increased in patients treated with DFP compared with DFO and in patients treated with combined DFP and DFO compared with DFO alone. People treated with all chelators must be kept under close medical supervision and treatment with DFP or DFX requires regular monitoring of neutrophil counts or renal function".

Unwanted effects with desferrioxamine

General tolerability and frequency of adverse effects

The unwanted effects of DFO are seen mainly when doses are given that are too high in relation to the level of iron overload, and typically take weeks or months to develop (over chelation). Some effects are largely independent of the dose given, however, limited data on the frequency of adverse effects at currently recommended doses are available, as most data were accumulated in the 1970s and 1980s, when optimal dosing was not fully understood. In a 1 year randomised clinical trial comparing DFO with DFX, abnormalities of hearing were reported as adverse events irrespective of drug relationship in 2.4% on DFO. Cataracts or lenticular opacities were reported as adverse events irrespective of drug relationship in 1.7% on DFO. A similar percentage of patients receiving DFX and DFO experienced cardiac adverse events (DFX 5.1%, DFO 6.9%).

Unwanted effects related to excessive chelation

- **Hearing problems**
  High frequency sensory neural loss, tinnitus and deafness may occur when DFO is given in high doses, particularly to young children whose iron burden is low (Olivieri 1986), and when the therapeutic index is exceeded (>0.025) (Porter 1989). Minor sensory neural deficit has been reversible in some cases, but significant hearing loss is usually permanent. Tinnitus may also occur. It is therefore advisable to monitor audiometry yearly, bearing in mind that audiometric changes due to excessive DFO are usually symmetrical; asymmetry suggests other pathology. Annual monitoring is particularly important in patients where SF values fall rapidly, or are <1000 µg/L, or in patients where the therapeutic index has been exceeded.

- **Effects on the eye**
  Visual disturbances are rare if dosage guidelines are not exceeded, and may include retinal effects and cataracts. Retinal effects were first noted when very high doses (>100 mg/kg/day) were given (Davies 1983). Symptoms may include night-blindness, impaired colour vision, impaired visual fields and reduced visual acuity. Severe cases may show signs of retinitis pigmentosa on fundoscopy, whereas milder cases are only demonstrable with electroretinography. Examination may also include scotoma and optic neuritis. The main risk factor appears to be high dosing (Olivieri 1986) but complications are also more likely in patients who have diabetes (Arden 1984), or those receiving concomitant phenothiazine treatment (Blake 1985). Treatment with DFO should be temporarily suspended in patients who develop complications, to be reintroduced at lower doses once investigations indicate resolution of the problem. Formal monitoring with electroretinography is recommended in patients on continuous DFO infusions, or in patients where high doses have been given relative to the iron load.

- **Growth retardation**
  Growth retardation may occur if DFO is administered at too high a dose. Another risk factor is a young age of starting treatment (<3 yrs) (De Virgillis 1988, Piga 1988). Growth velocity resumes rapidly when the dose is reduced to <40 mg/kg day, while it does not respond to hormonal treatment. It is therefore recommended that doses do not exceed 40 mg/kg until growth has ceased. Regular monitoring of growth is essential in all children (see Chapter 8 for details on endocrine complications).

- **Skeletal changes**
  Skeletal changes are more common in cases of excessive dosage of DFO, where patients have a low level of iron loading (Gabutti 1996, Olivieri 1992, De Virgillis 1988). Rickets-like bony lesions and genu valgum may be seen in association with metaphyseal changes, particularly in the vertebrae, giving a disproportionately short trunk. Radiographic features include vertebral demineralization and flatness of vertebral bodies. Patients should be regularly observed for such changes, as they are irreversible. Careful monitoring of growth charts for important toxic effects of DFO should be considered in the differential diagnosis if this falls away from previous growth curves.

- **Rare complications**
  Renal impairment may occur at high doses and renal monitoring is therefore recommended. Interstitial pneumonitis have been reported at very high doses of 10 mg/kg/h or more. Neurological complications have also been described; in patients without iron overload, DFO has induced reversible coma when used with a phenothiazine derivative (Blake 1985). Hypotension can also occur with rapid intravenous injection, as may occur during flushing of a line containing DFO, which should therefore be avoided.

Unwanted effects not related to excessive chelation

- **Local skin reactions**
  Reactions such as itching, erythema, induration and mild to moderate discomfort are common and may be due to inadequate dilution of DFO. Ulceration at the site of a recent infusion results from an intradermal infusion of DFO and should be addressed by deeper placement of the needle in subsequent infusions. The % solution infused should not exceed 10% or the risk of skin reactions increase.

- **Infection with Yersinia enterocolitica**
  This is an important risk associated with DFO treatment (described in detail in **Chapter 7**). Such infections may be difficult to diagnose. However, where there is reasonable clinical suspicion of infection of Yersinia enterocolitica, treatment with DFO should be temporarily discontinued. Infection should be considered in any patient with a febrile illness, especially when associated with abdominal pain, diarrhoea or joint pains, and should be treated as a medical emergency. DFO can usually be reintroduced once symptoms have subsided and a full course of antibiotics completed. Other infections such as Klebsiella may also be exacerbated by continued treatment with DFO. It is therefore recommended to cease administration of DFO in anyone with an unexplained fever, until the cause has been identified and effective antibiotic treatment begun. The decision as to when to recommence treatment with DFO requires clinical judgment and a careful balancing of the potential risks and benefits. For example, a patient with high cardiac iron or poor heart function may be at high risk if DFO is withheld during a septic episode, outweighing the risks of infection once antibiotics have been commenced.

- **Severe hypersensitivity**
  This is a rare event and can be treated by careful desensitization, carried out under close medical supervision (Bosquet 1983, Miller 1981). Desensitization is usually successful but may need to be repeated. If unsuccessful, an alternative chelator, such as DFP or DFX may be considered (see below).

Unwanted effects with DFP and their management

- **General tolerability and frequency of adverse effects**
  In clinical trials, the rates of adverse reactions based on pooled data collected from 642 patients who participated in single arm or active-controlled clinical studies are available. The most common were chromaturia (red in colour due to urine in iron), nausea (13%), vomiting, abdominal pain (10%), elevations in alanine aminotransferase (8%), arthralgia (10%) and neutropenia (7 %). Other unwanted effects >1% were back pain (2%), arthropathy (1%), agranulocytosis (1.7%) change in appetite (5%). diarrhea (3%), dyspepsia (2%) and headache (3%) (FDA 2011).

- **Relationship to dose and levels of iron overload**
  Most studies where tolerability has been reported have used 75 mg/kg in 3 divided doses. The drug is licensed up to 100 mg/kg/day but insufficient numbers have been reported to know whether the incidence of the most serious complication, namely agranulocytosis, is increased at these higher doses or indeed decreased at lower doses. Because there are no formal studies examining dose in relation to unwanted effects in DFP, it is not clear which of the effects described below are due to over-chelation and which are independent of this mechanism. These are therefore described together. As a result of the various unwanted effects, 20-30% of patients are unable to sustain long-term treatment with DFP (Hoffbrand 1998).

- **Neutropenia, agranulocytosis and thrombocytopenia**
  The labeling for DFP includes a boxed warning stating that the drug can cause agranulocytosis (absolute neutrophil count or ANC <500/mm3), which can lead to serious infections and death as a consequence of infection. This agranulocytosis may be preceded by neutropenia. The frequency reported incidence is approximately 1.7% of patients. Each patient's absolute neutrophil count should be measured before starting DFP therapy and weekly during treatment. The labeling states that DFP therapy should be interrupted and the patient's neutrophil count closely monitored if an infection develops. The mechanism for DFP's adverse effects on leukocytes is not known according to the labeling and is unpredictable in humans. Dose related effects on bone marrow hypoplasia and neutropenia has been seen in animal studies. Reported timing of onset of agranulocytosis is variable, from a few months to nine years. The condition may occur with thrombocytopenia, but isolated thrombocytopenia has also been occasionally reported, particularly in patients of Asian origin with probable hypersplenism.

  In a prospective trial where weekly neutrophil counts were undertaken and where DFP was discontinued when ANC was <1,500/mm3, agranulocytosis developed in 0.2/100 patient years and milder forms of neutropenia (ANC 500-1500/mm3) occurred in about 2.8/100 patient years (Cohen 2003, Cohen 2000). Neutropenia is more common in patients with intact spleens and commonly occurs in the first year of therapy. Recently, a continuation of DFP in patients with mild neutropenia (ANC 1000-1500/mm3) has been advocated by some investigators, with daily blood counts until resolution (El-Beshlawy 2013). However, it can be difficult in clinical practice to distinguish whether a low neutrophil count is part of a pattern of underlying neutropenia, or the first sign of agranulocytosis developing, which can be fatal. In 46 cases of agranulocytosis reported in Europe there were nine related deaths (Swedish Orphan 2006). Five cases were in patients who had been prescribed the drug for an unspecified 'off label' indications,

and several were not receiving weekly blood count monitoring. Swedish Orphan subsequently issued the following recommendations on the use of DFP: ''ANC should be monitored every week or more frequently if there are signs of infection; concomitant treatment that could affect the white cell count should be avoided; if severe neutropenia or agranulocytosis develop, the drug should be stopped and not reintroduced, and the use of GM CSF should be considered in the case of agranulocytosis; off-label use of the drug should be avoided''. Agranulocytosis and neutropenia has also been reported in patients taking combinations of DFP and DFO.

- **Gastrointestinal symptoms**
  Nausea, vomiting, gastric irritation and change in appetite (loss or gain) occurs in 3-24% of patients (Ceci 2002, Cohen 2000). A proportion of patients have to stop treatment for these reasons, which varies between studies. A liquid preparation is available which may be more palatable in children 1-10 years of age (ElAlfy 2010).

- **Effects on liver**
  In a summary of available clinical studies the FDA noted that 7.5% of 642 subjects developed increased ALT values. 0.62% of subjects discontinued the drug due to increased serum ALT levels, and 0.16% due to an increase in both ALT and AST (FDA, licensing information 2011). The frequency varied considerably between studies; about a quarter of patients show ALT fluctuations of twice the normal upper limit (Cohen 2000). By contrast, one prospective randomised study reported no significant end-of-study changes in liver enzymes with DFP or DFO (Pennell 2006b). An observational report of fibrosis after treatment of three or more years (Olivieri 1998) has not been supported by other reports (Wanless 2002, Hoffbrand, Tondury 1998). A relevant prospective randomised study investigating the progression to fibrosis using DFP for one year showed no difference as compared with DFO over the same period, and no difference in baseline and end-of-treatment liver function tests (Maggio 2002). A reduction of increased liver transaminase was observed in patients who were responding to DFP monotherapy, accompanied with a decreased LIC (Viprakasit 2013). Fluctuation of liver enzymes more than twice the upper limit of normal should prompt investigation of the cause and consideration for interrupting DFP therapy.

- **Arthropathy**
  The frequency of arthropathy varies greatly between studies, from as low as 4.5% at one year (Cohen 2000) to 15% after four years (Cohen 2003) in a predominantly European patient group, and as high as 33-40% in studies of patients from India (Sharma 2013, Choudhry 2004, Agarwal 1992). It is not yet clear whether these differences reflect environmental or genetic differences, or differences in iron overload between populations at the start of treatment. Symptoms range from mild non-progressive arthropathy, typically in the knees and controllable with non-steroidal anti-inflammatory drugs to (more rarely) severe erosive arthropathy that may progress even after treatment is stopped. Cases involving other joints, such as wrists, ankles and elbows and avascular necrosis of the hips have also been described. Radiologic evaluations revealed bony dysplasia, deformation and impaired growth of ulnar epiphyses in 13 out of 40 thalassaemia children who have received DFP for an average of 84 months (range 12-128 months) (Sharma 2013). It is recommended that treatment should be stopped where joint symptoms continue despite a reduction in DFP dose and are not controlled by non-steroidal anti-inflammatory drugs.

- **Neurological effects**
  DFP penetrates the blood brain barrier and has been evaluated for the treatment of conditions associated with brain iron deposition such as Freidrich's ataxia (Tsou 2009). Neurological complications are very rare in thalassaemia treatment and have been typically associated with unintentional overdosing (>230 mg/kg/day). Reports of rare neurological effects have included stroke, cognitive effects, nystagmus, walking disorders, ataxia, dystonia and impaired psychomotor skills. These effects appear to improve on cessation of treatment. Cerebellar syndrome has been described with combination therapy.

- **Effects on ears and eyes**
  One study reported continued audiometric deterioration after switching from DFO to DFP (Chiodo 1997). A recent study found hearing impairment and audiometric abnormalities in 56% of children with TM receiving DFP or DFO, with no difference between the two chelation groups, with the main shared risk factor low ferritin (Chao 2013). It may therefore be advisable to monitor audiometric function in patients on regimes containing DFP as well as DFO. There have been isolated reports of loss of vision (central scotoma). A regular eye examination including retinal evaluation at least once yearly is therefore advisable.

- **Other effects**
  Zinc deficiency has also been observed in some patients, especially those with diabetes (Al-Refaie 1994). Zinc deficiency is difficult to measure in plasma samples, and needs to be taken during fasting, in the absence of chelator in the blood. Zinc deficiency has been linked to toxicity of DFP in animal studies and were abrogated by zinc supplementation (Maclean 2001). Some clinicians routinely add zinc supplementation with DFP monotherapy or combination therapy is given (not given at the same time as the DFP) (Porter 2013b).

- **Frequency of adverse events compared with DFO**
  Adverse effects have been reported in four randomised studies comparing DFP with DFO. One trial has reported data that allows comparison of the probability of an adverse event with DFP and DFO (Maggio 2002), establishing a statistically significant two-fold difference between DFP (34%) and DFO (15%), but no difference between temporary or permanent treatment withdrawal.

- **Pregnancy**
  DFP is teratogenic in animals and must never be given to patients attempting to conceive. Until more is known, potentially fertile sexually active women and men taking DFP must use contraception. DFP should not be used in pregnant women.

- **Post marketing experience**
  A number of additional adverse reactions have been reported with post marketing experience (see highlights of prescribing information in section 6.2 (FDA 2011)). Because these are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or to establish a causal relationship to drug exposure.

Unwanted effects with DFX and their management

- **General tolerability and frequency of adverse effects**
  DFX has been given in the context of prospective trials to over 5900 patients with over 5 years of follow up in some prospective studies, so the relationship between DFX and unwanted effects are relatively well documented and defined. The frequency of adverse effects appears somewhat higher at doses of 25 to < 35 mg/k g/day (n = 136; 39.4%) than at 15 to < 25 mg/kg/day (n = 118; 31.1%) or < 15 mg/kg/day (n = 101; 29.4%).

- **Relationship to dose and iron overload**
  In general it is helpful to divide these effects into those which result from excess dosing with respect to the degree of iron overload and those which do not. Gastrointestinal, skin and renal effects can all be affected by dosing, although the exact relationship to body iron load has not been determined. It has however been shown in NTDT that with suitably low dosing (not exceeding 5-10 mg/kg) and low levels of ferritin or LIC, that low ferritin and LIC levels can be achieved without renal or other toxicities. Although a therapeutic index (as with DFO) has not been calculated, the general principle of reducing the dose, either as serum ferritin falls rapidly, or drops below 1000 µg/L for the first time would be adhered to. Although drug labelling suggests interrupting dosing when the serum ferritin reaches 500 µg/L, this leads to a 'stop-start' approach, which risks rebounding of NTBI and labile iron pools. Many clinicians therefore operate a dose reduction policy; giving very low doses (5 mg-10 mg/kg) to those patients who continue to be transfused. Some effects are notable by their absence, such as effects on growth, bone and arthropathy. The relationship of unwanted effects to iron load (LIC) has also been reported in 373 patients (Porter 2013a). Here, drug-related gastrointestinal AEs - mostly mild to moderate - were more frequently reported in patients with baseline LIC <7 versus those >/=7 mg/g dry wt, and were not confounded by diagnosis, dosing, ethnicity or a history of hepatitis B or C. Reported serum creatinine increases did not increase in low versus high-iron cohort patients. The smaller ESCALATOR trial however, found no clear trends in the type or frequency of drug-related AEs between the LIC <7 and ≥7 mg/g dry wt cohorts (Taher 2009). Tolerability of DFX tends to improve with long-term treatment (Cappellini 2011, Cappellini 2010). A practical guidance paper has been published which includes a summary of DFX -associated adverse events and a set of proposed management strategies (Vichinsky 2008).

- **Gastrointestinal effects**
  Gastrointestinal events are relatively frequent with DFX therapy but are typically mild to moderate and include diarrhoea, abdominal pain, nausea and vomiting, occurring in approximately 15-26% of patients (Vichinsky 2010). These symptoms rarely require dose adjustment or discontinuation, and decrease year on year over 5 years of follow up (Cappellini 2011). In the EPIC study, these symptoms were more common in patients with low baseline LIC values, as were abnormal LFTs (Porter 2013a). It is unclear to what extent the lactose component of the DFX formulation affects gastrointestinal tolerability in lactose-intolerant patients but this requires clarification, as lactose intolerance is common - particularly in South-East Asia. The role of co-administration of acidophilus or lactobacillus probiotic yoghurt to aid lactose has not been systematically studied. Hematemesis and melena due to gastric and/or duodenal ulceration has been reported in patients taking DFX (Yadav 2013, Bauters 2010). These patients should be investigated and managed appropriately, including Helicobacter pylori eradication therapy if

required. Special attention should be taken in patients taking concomitant medications that can increase the possibility of gastric ulceration. Although the manufacturer does not recommend taking DFX with food or dividing the doses, some clinical experience has found that more flexible dosing schedules may be more suitable in patients who experience gastrointestinal disturbances. While the drug should still be taken at the same time each day, administration of DFX in the evening with or after food can potentially improve gastrointestinal tolerability and compliance. For example, a recent study found that palatability and gastrointestinal tolerability of DFX was improved when patients were allowed to take treatment with a soft food at breakfast, or with a beverage of choice (Goldberg 2013). Another option is to divide the DFX dose, which can help to reduce gastrointestinal events without adversely affecting iron excretion (Otto-Duessel 2007).

- **Skin rashes**

  Skin rashes occurred in 7-11% of patients, and were typically pruritic, maculopapular and generalised, but occasionally confined to palms and soles of the feet. Skin rash is more common in Asian population (up to 18%), often mild in severity and rarely developing into severe drug-hypersensitivity (Viprakasit 2011). Rash typically develops within two weeks of starting treatment. A minority of patients require permanent discontinuation of therapy, and mild rashes often resolve without dose modification, and became very rare after year 1 of treatment (Cappellini 2011). For moderate to severe rashes, treatment should be stopped and later restarted at a very low dose (<5 mg/kg), slowly increasing to therapeutic doses. Severe skin rash associated with angioedema is rare, and unlike the more common rash seen with DFX, may not respond to interruption and reintroduction at a lower dose. Here, DFX therapy may need to be halted completely as the angioedema may be evidence of an immune sensitisation response.

- **Renal effects**

- An increase in serum creatinine ≥30% on at least two consecutive readings was observed in 38% of patients receiving DFX, most frequently at doses of 20 mg/kg and 30 mg/kg (Cappellini 2006). These increases were sometimes transient and generally within the normal ranges, never exceeding two times the upper limit of normal (ULN), and were more frequent in the population of patients having the most dramatic decrease in LIC and serum ferritin. In a randomized study, dose reduction of 33-50% was planned if at least two consecutive increases in serum creatinine were >33% above baseline. As the creatinine spontaneously normalised in a number of cases, dose reductions were instituted in only 13%. In about 25% of those cases, the creatinine then returned to baseline, while in the rest it remained stable or fluctuated between baseline and the maximum increase observed prior to dose reduction. At 5 years of follow up, no evidence of progressive renal dysfunction had been reported where the above doses and modifications were used (Cappellini 2011). Other causes of increasing creatinine should also be considered in patients on DFX therapy, such as renal stones or concomitant use of NSAIDs. If a patient becomes acutely unwell for another reason, such as septicaemic shock or severe acute vaso-occlusive complications in SCD, it is probably wise to interrupt chelation therapy until the general condition stabilises.

- Proteinuria may be present in about a quarter of thalassaemia major patients, irrespective of the underlying chelation therapy, with average values about three times that of healthy controls (Economou 2010). Elevation of urine calcium and cystatin C are

also seen in patients on DFX or DFP and DFO, whereas elevation of B2microglobulin was seen in patients on DFX only (Economou 2010). It is recommended that urine is monitored regularly for protein, and this can be conveniently performed at the time of visits for cross matching blood. Although proteinuria can fluctuate considerably, if there is a clear upward trend in the protein/creatinine ration above 1 mg/g, interruption or dose reduction should be considered. Current drug labelling recommends monthly urine testing for protein, which is helpful in establishing trends in proteinuria, as isolated estimates can be misleading.

- Case reports of renal tubular acidosis (Fanconi syndrome) with electrolyte imbalance, and metabolic acidosis due to tubular dysfunction have been rarely reported in adults and children taking DFX (Rheault 2011, Grange 2010). All cases recovered following withdrawal of DFX and appropriate electrolyte supplementation. Symptoms of renal tubular acidosis can be non-specific but may include polyuria, polydipsia and dehydration. Investigations may show proteinuria, hypokalemia, hypophosphatemia, hyperchloremic metabolic acidosis with excessive loss of substances in the urine (e.g. amino acids, glucose, phosphate and bicarbonate). Some patients, especially children, have intercurrent infections associated with Fanconi syndrome. Renal impairment due to DFX may also develop as part of a generalized delayed hypersensitivity reaction. It is recommended that kidney and proximal tubular function be periodically monitored in patients receiving DFX throughout their course of therapy.

- **Hepatic effects**
  Generally speaking liver enzymes improve in line with falling LIC. However, increases in liver transaminases are occasionally seen. In the EPIC study 0.6% of 1115 TM patients showed an increase of AST >10x the upper limit of normal (Cappellini 2010). These changes are commonly observed within 1 month of initiating DFX therapy, although this may occur later, particularly at high doses in patients with low iron burdens. Careful baseline assessment of alanine transaminase levels (ALT) should therefore be performed before starting treatment, and monitored every two weeks for the first month. Thereafter, checking ALT approximately monthly is recommended. Abnormal liver function tests are more frequent in children receiving DFX, and in such instances chelation should be stopped and ALT levels carefully monitored to ensure they return to normal. Reintroducing DFX using a slow escalation schedule has been reported in such cases, where the patient is started on DFX 125 mg, with the dose increased every 2–3 weeks provided the ALT levels remain stable with weekly monitoring. Improvements in the liver pathology of 219 patients with beta-thalassaemia treated with DFX for at least 3 years has been reported in a prospective trial (Deugnier 2011). By the end of the study, stability of Ishak fibrosis staging scores (change of -1, 0, or +1) or improvements (change of </=-2) were observed in 82.6% of patients. DFX treatment for 3 or more years reversed or stabilized liver fibrosis in 83% of patients with iron-overloaded beta-thalassaemia.

- **Effects on hematology**
  Although hematological adverse effects such as thrombocytopenia and agranulocytosis have been added to the product information since DFX was approved, only a few cases have been published as case reports thus far. In two patients, thrombocytopenia due to DFX developed as part of a generalized delayed hypersensitivity reaction, in combination with a rash, fever, eosinophilia, hepatic and renal impairment (Wei 2011, Gutiérrez Macías 2010).

- **Arthropathy and growth failure**
  No cases of arthropathy or growth failure have been associated with DFX administration. Comparing 296 patients who received DFX in a one-year prospective randomised study with 290 patients receiving DFO, deafness, neurosensory deafness or hypoacusis were reported as adverse events in eight patients on DFX, and seven on DFO.

- **Eyes and ears**
  These are very rare and their significance is uncertain, however, current labelling recommends yearly auditory and eye assessments (Novartis 2013). Early lens opacity was reported in the DFX core registration trials, but the incidence (0.3%) did not significantly differ from the control group of DFO treated patients (Cappellini 2006). The electroretinographic effects previously seen with DFO have not been described, and the frequency at which electroretinography assessment is indicated has not been formally assessed. Possible audiometric effects were identified in early studies but this has not been reported systematically. One investigator has reported lens opacities in 3 out of 12 patients (Bloomfield 1978), which would approximate to 80-times the incidence observed in the large-scale trials, the reason for which is presently unclear (Ford 2008).

- **Pregnancy and DFX**
  DFX has been shown to have teratogenic effects in animal studies. However, there has been a report of a healthy male baby delivered to a 38-year-old mother with thalassemia major who has unintentionally conceived during DFX therapy (Anastasi 2011). However, at present it is recommended that thalassaemia patients who plan to conceive should avoid the use of iron chelation for at least 3 months before.

- **Post marketing experience**
  A number of additional adverse reactions have been reported with post marketing experience (see highlights of prescribing information in section 6.2 of (FDA 2011)). Because these are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or to establish a causal relationship to drug exposure.

Appendix 3

Practical Issues with DFO Infusions

Practical issues with subcutaneous infusion
Because regular use of DFO is critical to a good outcome, every effort should be made with each individual to help him or her to find the most convenient way to infuse the drug.

- **Strength of infusion**
  The manufacturers of DFO recommend that each 500 mg vial of the drug is dissolved in at least 5 ml of water, giving a 10% solution. Concentrations in excess of this may increase the risk of local reactions at the site of infusion.

- **Site of infusion**
  Care must be taken to avoid inserting needles near important vessels, nerves or organs. The abdomen is generally the best place. However, because of local reactions such as erythema, swelling and induration, it is often necessary to 'rotate' the sites used for

injection (see **Figure 3**). Some patients find that the skin over the deltoid or the lateral aspect of the thigh provides useful additional, alternative sites. The best needle to use will depend on the individual. Many patients are happy with butterfly needles of 25 gauge or smaller, which are inserted at an angle of about 45 degrees to the skin surface. The needle tip should move freely when the needle is waggled. Other patients prefer needles that are inserted vertically through the skin and are fixed with an adhesive tape attached to the needle (see **Figure 4**). Patient preference is highly variable and clinicians should explore the best type of needle for each patient, to help maximize compliance.

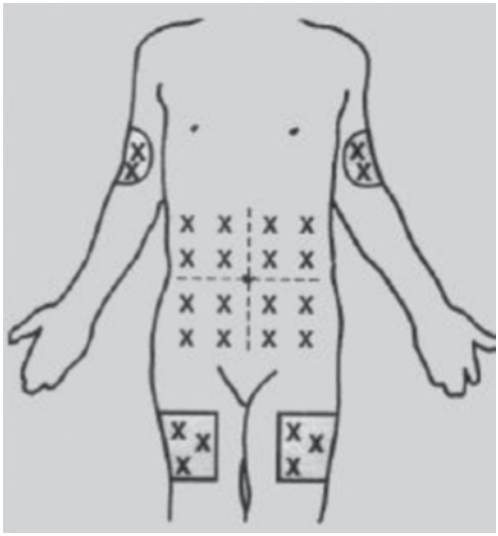

Figure 3. Rotation of infusion sites.

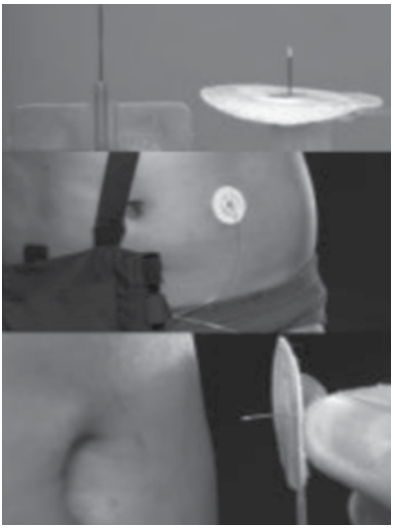

Figure 4. Insertion of needles for desferrioxamine infusions.

- **Types of infuser**
  There are many types of infuser now available. Newer devices, including balloon pumps, are smaller, lighter, and quieter than their predecessors. For patients who find dissolving, mixing and drawing up DFO a problem, pre-filled syringes or balloons may be useful. Some pumps are designed to monitor compliance.

- **Local reactions**
  Persistent local reactions may be reduced by varying injection sites, lowering the strength of infusion, or in severe cases, by adding 5-10 mg of hydrocortisone to the infusion mixture. Application of topical low potency corticosteroid cream after injection can reduce local reactions.

Practical details for intravenous infusions

10 % solutions of DFO given to peripheral veins will damage and sclerose the vein. If infused (as an emergency) into a peripheral vein, the solution must be diluted – for example in 200-500 mls of saline.

- **Management of in-dwelling intravenous lines**
  Infection and thrombosis of catheters may occur. Careful aseptic procedures must be followed in order to prevent possible infection by Staphylococcus epidermidis and aureus, which when established are difficult to eradicate, and often removal of the infusion system becomes necessary. The risk of thrombosis and infection is likely to be greater in centres that do not have regular experience in the use of long-term in-dwelling lines (Piga 2006). Use of prophylactic anticoagulation is advised, as line-thrombosis is relatively common in thalassaemia major (Davis 2000). As development of thrombosis can occur at the tip of the catheter, it is advisable if possible to avoid placing the tip in the right atrium.

- **Intravenous DFO with blood transfusion**
  This has been used as a supplement to conventional therapy (e.g. 1g over 4 hours piggy-backed into the infusion line), but its contribution to iron balance is very limited and not recommended as a standard procedure. Special attention must be given to avoiding accidental boluses due to DFO collecting in the dead space of the infusion line. Co-administration of DFO and blood can lead to errors in interpreting side effects, such as acute fever, rashes, anaphylaxis and hypotension during blood transfusion. DFO should never be added directly into the blood unit.

- **Use of DFO by subcutaneous bolus**
  If an infusion pump is not available or if 10-hour infusions are not tolerated, bolus subcutaneous treatment may be considered if the patient is not at high risk of heart disease. A randomised study has shown that serum ferritin and liver iron can be controlled equally effectively by giving an equivalent total dose (45 mg/kg x 5 per week) either as two subcutaneous 'boluses' or as a nightly 10-hour subcutaneous infusion (Yarali 2006). However, this technique may be impractical in the clinic – particularly in paediatric patients, due to the painful nature of bolus infusions.

References

Adamkiewicz TV, Abboud MR, Paley C, et al. Serum ferritin level changes in children with sickle cell disease on chronic blood transfusion are nonlinear and are associated with iron load and liver injury. Blood 2009;114:4632-8.

Agarwal MB, Gupte SS, Viswanathan C, et al. Long-term assessment of efficacy and safety of L1, an oral iron chelator, in transfusion dependent thalassaemia: Indian trial. Br J Haematol 1992;82:460-6.

Al-Refaie FN, Wonke B, Hoffbrand AV, et al. Efficacy and possible adverse effects of the oral iron chelator 1,2-dimethyl-3-hydroxypyridin-4-one (L1) in thalassemia major. Blood 1992;80:593-9.

Al-Refaie FN, Wonke B, Wickens DG, et al. Zinc concentration in patients with iron overload receiving oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one or desferrioxamine. J Clin Pathol 1994;47:657-60.

Aldouri MA, Hoffbrand AV, Flynn DM, et al. High incidence of cardiomyopathy in beta-thalassemia patients receiving transfusion and iron chelation: reversal by intensified chelation. Acta Haematologica 1990;84:113-7.

Anastasi S, Lisi R, Abbate G, et al. Absence of teratogenicity of deferasirox treatment during pregnancy in a thalassaemic patient. Pediatr Endocrinol Rev 2011;8 Suppl 2:345-7.

Anderson LJ, Holden S, Davis B, et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. Eur Heart J 2001;22:2171-9.

Anderson LJ, Westwood MA, Holden S, et al. Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: a prospective study using T2* cardiovascular magnetic resonance. Br J Haematol 2004;127:348-55.

Ang AL, Shah F, Davis B, et al. Deferiprone Is Associated with Lower Serum Ferritin (SF) Relative to Liver Iron Concentration (LIC) Than Deferoxamine and Deferasirox-Implications for Clinical Practice (abstract). Blood 2010;116:4246.

Angelucci E, Brittenham GM, McLaren CE, et al. Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med 2000;343:327-31.

Angelucci E, Giovagnoni A, Valeri G, et al. Limitations of magnetic resonance imaging in measurement of hepatic iron. Blood 1997;90:4736-42.

Au WY, Lam WW, Chu WW, et al. A cross-sectional magnetic resonance imaging assessment of organ specific hemosiderosis in 180 thalassemia major patients in Hong Kong. Haematologica 2008;93:784-6.

Aydinok Y, Kattamis A, Cappellini MD, et al. Deferasirox–Deferoxamine Combination Therapy Reduces Cardiac Iron With Rapid Liver Iron Removal In Patients With Severe

Transfusional Iron Overload (HYPERION)(abstract). Blood 2013;2257.

Ayidinok Y, Kattamis A, Cappellini MD, et al. Deferasirox-Deferoxamine combination therapy reduces cardiac iron with rapid liver iron removal after 24 months in patients with severe transfusional iron overload (HYPERION) (abstract). Haematologica 2014;99:229.

Aydinok Y, Evans P, Manz CY, et al. Timed non-transferrin bound iron determinations probe the origin of chelatable iron pools during deferiprone regimens and predict chelation response. Haematologica 2012a;97:835-41.

Aydinok Y, Ulger Z, Nart D, et al. A randomized controlled 1-year study of daily deferiprone plus twice weekly desferrioxamine compared with daily deferiprone monotherapy in patients with thalassemia major. Haematologica 2007;92:1599-606.

Aydinok Y, Unal S, Oymak Y, et al. Observational study comparing long-term safety and efficacy of Deferasirox with Desferrioxamine therapy in chelation-naive children with transfusional iron overload. Eur J Haematol 2012b;88:431-8.

Bauters T, Mondelaers V, Robays H, et al. Gastric ulcer in a child treated with deferasirox. Pharm World Sci 2010;32:112-3.

Blake DR, Winyard P, Lunec J, et al. Cerebral and ocular toxicity induced by desferrioxamine. Quarterly Journal of Medicine 1985;56:345-55.

Bloomfield SE, Markenson AL, Miller DR, et al. Lens opacities in thalassemia. J Pediatr Ophthalmol Strabismus 1978;15:154-6.

Borgna-Pignatti C, Cappellini MD, De Stefano P, et al. Cardiac morbidity and mortality in deferoxamine- or deferiprone-treated patients with thalassemia major. Blood 2006;107:3733-7.

Borgna-Pignatti C, Rugolotto S, De Stefano P, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica 2004;89:1187-93.

Bosquet J, Navarro M, Robert G, et al. Rapid desensitisation for desferrioxamine anaphylactoid reaction. Lancet 1983;2:859-60.

Brittenham GM, Cohen AR, McLaren CE, et al. Hepatic iron stores and plasma ferritin concentration in patients with sickle cell anemia and thalassemia major. Am J Hematol 1993;42:81-5.

Brittenham GM, Griffith PM, Nienhuis AW, et al. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major [see comments]. N Engl J Med 1994;331:567-73.

Bronspiegel-Weintrob N, Olivieri NF, Tyler B, et al. Effect of age at the start of iron chelation therapy on gonadal function in beta-thalassemia major. N Engl J Med 1990;323:713-9.

Buja LM and Roberts WC. Iron in the heart. Etiology and clinical significance. Am J Med 1971;51:209-21.

Cabantchik ZI, Breuer W, Zanninelli G, et al. LPI-labile plasma iron in iron overload. Best Pract Res Clin Haematol 2005;18:277-87.

Cappellini MD, Bejaoui M, Agaoglu L, et al. Iron chelation with deferasirox in adult and pediatric patients with thalassemia major: efficacy and safety during 5 years' follow-up. Blood 2011;118:884-93.

Cappellini MD, Bejaoui M, Agaoglu L, et al. Prospective evaluation of patient-reported outcomes during treatment with deferasirox or deferoxamine for iron overload in patients with beta-thalassemia. Clin Ther 2007;29:909-17.

Cappellini MD, Cohen A, Piga A, et al. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. Blood 2006;107:3455-62.
Cappellini MD, Porter J, El-Beshlawy A, et al. Tailoring iron chelation by iron intake and serum ferritin: the prospective EPIC study of deferasirox in 1744 patients with transfusion-dependent anemias. Haematologica 2010;95:557-66.

Carpenter JP, He T, Kirk P, et al. On T2* magnetic resonance and cardiac iron. Circulation 2011;123:1519-28.

Ceci A, Baiardi P, Felisi M, et al. The safety and effectiveness of deferiprone in a large-scale, 3-year study in Italian patients. British journal of haematology 2002;118:330-6.

Chao YH, Wu KH, Lin CY, et al. Audiologic and vestibular assessment in patients with beta-thalassemia major receiving long-term transfusion therapy. Pediatr Blood Cancer 2013;60:1963-6.

Chatterjee R, Katz M, Oatridge A, et al. Selective loss of anterior pituitary volume with severe pituitary- gonadal insufficiency in poorly compliant male thalassemic patients with pubertal arrest. Ann N Y Acad Sci 1998;850:479-82.

Chiodo AA, Alberti PW, Sher GD, et al. Desferrioxamine ototoxicity in an adult transfusion-dependent population. J Otolaryngol 1997;26:116-22.

Choudhry VP, Pati HP, Saxena A, et al. Deferiprone, efficacy and safety. Indian J Pediatr 2004;71:213-6.

Cohen AR, Galanello R, Piga A, et al. Safety and effectiveness of long-term therapy with the oral iron chelator deferiprone. Blood 2003;102:1583-7.

Cohen AR, Galanello R, Piga A, et al. Safety profile of the oral iron chelator deferiprone: a multicentre study. Br J Haematol 2000;108:305-12.
Cohen AR, Glimm E and Porter JB. Effect of transfusional iron intake on response to chelation therapy in beta-thalassemia major. Blood 2008;111:583-7.

Cohen AR, Mizanin J and Schwartz E. Rapid removal of excessive iron with daily, high-dose intravenous chelation therapy. J Pediatr 1989;115:151-5.

Daar S, Pathare A, Nick H, et al. Reduction in labile plasma iron during treatment with deferasirox, a once-daily oral iron chelator, in heavily iron-overloaded patients with beta-thalassaemia. Eur J Haematol 2009;82:454-7.Davies SC, Marcus RE, Hungerford JL, et al. Ocular toxicity of high-dose intravenous desferrioxamine. Lancet 1983;2:181-4.

Davis B, O'Sullivan C and Porter J. Value of LVEF monitoring in the long-term management of beta-thalassaemia. 8th International Conference on Thalassemia and the hemoglobinopathies (Athens) 2001;Abstract 056:147.

Davis BA, O'Sullivan C, Jarritt PH, et al. Value of sequential monitoring of left ventricular ejection fraction in the management of thalassemia major. Blood 2004;104:263-9.

Davis BA and Porter JB. Long-term outcome of continuous 24-hour deferoxamine infusion via indwelling intravenous catheters in high-risk beta-thalassemia. Blood 2000;95:1229-36.

De Virgillis S, Congia M, Frau F, et al. Desferrioxamine-induced growth retardation in patients with thalassaemia major. Journal of Pediatrics 1988; 113:661-9.

Del Vecchio GC, Schettini F, Piacente L, et al. Effects of deferiprone on immune status and cytokine pattern in thalassaemia major. Acta Haematol 2002;108:144-9.

Deugnier Y, Turlin B, Ropert M, et al. Improvement in liver pathology of patients with beta-thalassemia treated with deferasirox for at least 3 years. Gastroenterology 2011;141:1202-11, 11 e1-3.

Economou M, Printza N, Teli A, et al. Renal dysfunction in patients with beta-thalassemia major receiving iron chelation therapy either with deferoxamine and deferiprone or with deferasirox. Acta haematologica 2010;123:148-52.

El-Beshlawy A, Manz C, Naja M, et al. Iron chelation in thalassemia: combined or monotherapy? The Egyptian experience. Ann Hematol 2008;87:545-50.

Elalfy M, Wali Y, Tony S, et al. Comparison Of Two Combination Iron Chelation Regimens, Deferiprone and Deferasirox Versus Deferiprone and Deferoxamine, In Pediatric Patients With ß-Thalassemia Major (abstract). Blood 2013;22:559.

ElAlfy MS, Sari TT, Lee CL, et al. The safety, tolerability, and efficacy of a liquid formulation of deferiprone in young children with transfusional iron overload. J Pediatr Hematol Oncol 2010;32:601-5.

Evans P, Kayyali R, Hider RC, et al. Mechanisms for the shuttling of plasma non-transferrin-bound iron (NTBI) onto deferoxamine by deferiprone. Transl Res 2010;156:55-67.

Farmaki K, Tzoumari I, Pappa C, et al. Normalisation of total body iron load with very intensive combined chelation reverses cardiac and endocrine complications of thalassaemia major. Br J Haematol 2010;148:466-75.

FDA drug prescribing information for ferriprox 2011. Available online at: www.accessdata.fda.gov/drugsatfda_docs/label/2011/021825lbl.pdf 2011;

Filosa A, Vitrano A, Rigano P, et al. Long-term treatment with deferiprone enhances left ventricular ejection function when compared to deferoxamine in patients with thalassemia major. Blood Cells Mol Dis 2013;51:85-8.

Fischer R, Longo F, Nielsen P, et al. Monitoring long-term efficacy of iron chelation therapy by deferiprone and desferrioxamine in patients with beta-thalassaemia major: application of SQUID biomagnetic liver susceptometry. Br J Haematol 2003;121:938-48.

Fischer R, Piga A, De Sanctis V, et al. Large-Scale Study in Thalassemia Using Biomagnetic Liver Susceptometry. Biomag 98: Proc. 11th Inte. Conf. Biomagnetism (Sendai) 1998;1-4.

Fisher CA, Premawardhena A, de Silva S, et al. The molecular basis for the thalassaemias in Sri Lanka. British journal of haematology 2003;121:662-71.

Fisher SA, Brunskill SJ, Doree C, et al. Oral deferiprone for iron chelation in people with thalassaemia. Cochrane Database Syst Rev 2013a;8:CD004839.

Fisher SA, Brunskill SJ, Doree C, et al. Desferrioxamine mesylate for managing transfusional iron overload in people with transfusion-dependent thalassaemia. Cochrane Database Syst Rev 2013b;8:CD004450.

Ford J and Rojkjaer L. Comment to: Development of lens opacities with peculiar characteristics in patients affected by thalassemia major on chelating treatment with deferasirox. Haematologica 2008; 2008;93:e49.

Freeman AP, Giles RW, Berdoukas VA, et al. Early left ventricular dysfunction and chelation therapy in thalassemia major. Ann Intern Med 1983;99:450-4.

Gabutti V and Piga A. Results of long-term iron-chelating therapy. Acta Haematol 1996;95:26-36.

Galanello R, Kattamis A, Piga A, et al. A prospective randomized controlled trial on the safety and efficacy of alternating deferoxamine and deferiprone in the treatment of iron overload in patients with thalassemia. Haematologica 2006;91:1241-3.

Galanello R, Piga A, Alberti D, et al. Safety, tolerability, and pharmacokinetics of ICL670, a new orally active iron-chelating agent in patients with transfusion-dependent

iron overload due to beta-thalassemia. J Clin Pharmacol 2003;43:565-72.

Galanello R, Piga A, Cappellini MD, et al. Effect of food, type of food, and time of food intake on deferasirox bioavailability: recommendations for an optimal deferasirox administration regimen. J Clin Pharmacol 2008;48:428-35.

Garbowski M, Carpenter J-P, Smith G, et al. Calibration of Improved T2* Method for the Estimation of Liver Iron Concentration in Transfusional Iron Overload (abstract). Blood 2009;114:2004.

Goldberg SL, Giardina PJ, Chirnomas D, et al. The palatability and tolerability of deferasirox taken with different beverages or foods. Pediatr Blood Cancer 2013;60:1507-12.

Gomber S, Saxena R and Madan N. Comparative efficacy of desferrioxamine, deferiprone and in combination on iron chelation in thalassemic children. Indian Pediatr 2004;41:21-7.

Gosriwatana I, Loreal O, Lu S, et al. Quantification of non-transferrin-bound iron in the presence of unsaturated transferrin. Anal Biochem 1999;273:212-20.

Grange S, Bertrand DM, Guerrot D, et al. Acute renal failure and Fanconi syndrome due to deferasirox. Nephrol Dial Transplant 2010;25:2376-8.

Gutiérrez Macías A, Lizarralde Palacios E, Olabarría Santurtún I, et al. Sindrome de hipersensibilidad retardada inducido por deferasirox [Deferasirox-induced delayed hypersensitivity syndrome]. Med Clin (Barc) 2010;134:329-30.

Ha SY, Chik KW, Ling SC, et al. A randomized controlled study evaluating the safety and efficacy of deferiprone treatment in thalassemia major patients from Hong Kong. Hemoglobin 2006;30:263-74.

Hod EA, Zhang N, Sokol SA, et al. Transfusion of red blood cells after prolonged storage produces harmful effects that are mediated by iron and inflammation. Blood 2010;115:4284-92.

Hoffbrand AV, F AL-R, Davis B, et al. Long-term trial of deferiprone in 51 transfusion-dependent iron overloaded patients. Blood 1998;91:295-300.

Jensen PD, Jensen FT, Christensen T, et al. Evaluation of myocardial iron by magnetic resonance imaging during iron chelation therapy with deferrioxamine: indication of close relation between myocardial iron content and chelatable iron pool. Blood 2003;101:4632-9.

Jensen PD, Jensen FT, Christensen T, et al. Non-invasive assessment of tissue iron overload in the liver by magnetic resonance imaging. Br J Haematol 1994;87:171-84.

Kattamis A, Ladis V, Berdousi H, et al. Iron chelation treatment with combined therapy with deferiprone and deferioxamine: a 12-month trial. Blood Cells Mol Dis 2006;36:21-5.

Kirk P, He T, Anderson LJ, et al. International reproducibility of single breathhold T2* MR for cardiac and liver iron assessment among five thalassemia centers. J Magn Reson Imaging 2010;32:315-9.

Kirk P, Roughton M, Porter JB, et al. Cardiac T2* magnetic resonance for prediction of cardiac complications in thalassemia major. J. of Cardiovasr Mag Res 2009a;1(Suppl 1):O2:1-2.

Kirk P, Roughton M, Porter JB, et al. Cardiac T2* magnetic resonance for prediction of cardiac complications in thalassemia major. Circulation 2009b;120:1961-8.

Kontoghiorghes GJ, Goddard JG, Bartlett AN, et al. Pharmacokinetic studies in humans with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one. Clinincal Pharmacology 1990;48:255-61.

Lal A, Porter J, Sweeters N, et al. Combined chelation therapy with deferasirox and deferoxamine in thalassemia. Bld cells, molecules & diseases 2013;50:99-104.

Limenta LM, Jirasomprasert T, Jittangprasert P, et al. Pharmacokinetics of deferiprone in patients with beta-thalassaemia: impact of splenectomy and iron status. Clin Pharmacokinet 2011;50:41-50.

Maclean KH, Cleveland JL and Porter JB. Cellular zinc content is a major determinant of iron chelator-induced apoptosis of thymocytes. Blood 2001;98:3831-9.

Maggio A, D'Amico G, Morabito A, et al. Deferiprone versus deferoxamine in patients with thalassemia major: a randomized clinical trial. Blood Cells Mol Dis 2002;28:196-208.

Maggio A, Filosa A, Vitrano A, et al. Iron chelation therapy in thalassemia major: A systematic review with meta-analyses of 1520 patients included on randomized clinical trials. Bld cells, molecules & diseases 2011;47:166-75.

Maggio A, Vitrano A, Capra M, et al. Long-term sequential deferiprone-deferoxamine versus deferiprone alone for thalassaemia major patients: a randomized clinical trial. British Journal of Haematology 2009;145:245-54.

Maggio A, Vitrano A, Lucania G, et al. Long-term use of deferiprone significantly enhances left-ventricular ejection function in thalassemia major patients. American journal of hematology 2012;87:732-3.

Marcus RE, Davies SC, Bantock HM, et al. Desferrioxamine to improve cardiac function in iron overloaded patients with thalassaemia major. Lancet 1984;1:392-3.

Miller JM, Horger EO, Key TC, et al. Management of sickle hemoglobinopathies in pregnant patients. Obstetrics & Gynecology 1981;141:237-41.

Modell B, Khan M and Darlison M. Survival in beta-thalassaemia major in the UK: data from the UK Thalassaemia Register. Lancet 2000;355:2051-2.

Mourad FH, Hoffbrand AV, Sheikh-Taha M, et al. Comparison between desferrioxamine and combined therapy with desferrioxamine and deferiprone in iron overloaded thalassaemia patients. Br J Haematol 2003;121:187-9.

Nisbet-Brown E, Olivieri NF, Giardina PJ, et al. Effectiveness and safety of ICL670 in iron-loaded patients with thalassaemia: a randomised, double-blind, placebo-controlled, dose-escalation trial. Lancet 2003;361:1597-602.

Noetzli LJ, Carson SM, Nord AS, et al. Longitudinal analysis of heart and liver iron in thalassemia major. Blood 2008;112:2973-8.
Noetzli LJ, Papudesi J, Coates TD, et al. Pancreatic iron loading predicts cardiac iron loading in thalassemia major. Blood 2009;114:4021-6.

Novartis 2013. Summary of Product Characteristics. Available online at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR__Product_Information/human/000670/WC500033925.pdf. 2013;

Olivieri N and Brittenham G. Final Results of the randomised trial of deferiprone and deferoxamine. Blood 1997;90:264a.

Olivieri NF, Brittenham GM, Matsui D, et al. Iron-chelation therapy with oral deferiprone in patients with thalassemia major [see comments]. N Engl J Med 1995;332:918-22.

Olivieri NF, Brittenham GM, McLaren CE, et al. Long-term safety and effectiveness of iron-chelation therapy with deferiprone for thalassemia major. The New England journal of medicine 1998a;339:417-23.

Olivieri NF, Buncic JR, Chew E, et al. Visual and auditory neurotoxicity in patients receiving subcutaneous deferoxamine infusions. N Engl J Med 1986;314:869-73.

Olivieri NF, Koren G, Harris J, et al. Growth failure and bony changes induced by deferoxamine. American Journal of Pediatric Hematology Oncology 1992;14:48-56.

Olivieri NF, Nathan DG, MacMillan JH, et al. Survival in medically treated patients with homozygous beta-thalassemia. New England Journal of Medicine 1994;331:574-8.

Origa R, Bina P, Agus A, et al. Combined therapy with deferiprone and desferrioxamine in thalassemia major. Haematologica 2005;90:1309-14.

Otto-Duessel M, Aguilar M, Nick H, et al. Comparison of twice-daily vs once-daily deferasirox dosing in a gerbil model of iron cardiomyopathy. Exp Hematol 2007;35:1069-73.

Pennell D. MRI and iron-overload cardiomyopathy in thalassaemia. Circulation 2006a;113:f43-4.

Pennell DJ, Berdoukas V, Karagiorga M, et al. Randomized controlled trial of deferiprone or deferoxamine in beta-

thalassemia major patients with asymptomatic myocardial siderosis. Blood 2006b;107:3738-44.

Pennell DJ, Porter JB, Cappellini MD, et al. Deferasirox for up to 3 years leads to continued improvement of myocardial T2* in patients with beta-thalassemia major. Haematologica 2012;97:842-8.

Pennell DJ, Porter JB, Cappellini MD, et al. Efficacy of deferasirox in reducing and preventing cardiac iron overload in beta-thalassemia. Blood 2010;115:2364-71.

Pennell DJ, Porter JB, Piga A, et al. A 1-year randomized controlled trial of deferasirox versus deferoxamine for myocardial iron removal in beta-thalassemia major (CORDELIA). Blood 2014;123:1447-1454.

Pepe A, Meloni A, Capra M, et al. Deferasirox, deferiprone and desferrioxamine treatment in thalassemia major patients: cardiac iron and function comparison determined by quantitative magnetic resonance imaging. Haematologica 2011;96:41-7.

Pepe A, Meloni A, Rossi G, et al. Cardiac and hepatic iron and ejection fraction in thalassemia major: multicentre prospective comparison of combined deferiprone and deferoxamine therapy against deferiprone or deferoxamine monotherapy. Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance 2013;15:1.

Piga A, Galanello R, Forni GL, et al. Randomized phase II trial of deferasirox (Exjade, ICL670), a once-daily, orally-administered iron chelator, in comparison to deferoxamine in thalassemia patients with transfusional iron overload. Haematologica 2006;91:873-80.

Piga A, Longo F, Duca L, et al. High nontransferrin bound iron levels and heart disease in thalassemia major. Am J Hematol 2009;84:29-33.

Piga A, Luzzatto L, Capalbo P, et al. High dose desferrioxamine as a cause of growth failure in thalassaemic patients. European Journal Haematology 1988;40: 380-1.

Pippard MJ, Callender ST and Finch CA. Ferrioxamine excretion in iron-loaded man. Blood 1982;60:288-94.

Pongtanakul B and Viprakasit V. Twice daily deferasirox significantly improves clinical efficacy in transfusion dependent thalassaemias who were inadequate responders to standard once daily dose. Blood Cells Mol Dis 2013;51:96-7.

Porter J, Bowden DK, Economou M, et al. Health-Related Quality of Life, Treatment Satisfaction, Adherence and Persistence in beta-Thalassemia and Myelodysplastic Syndrome Patients with Iron Overload Receiving Deferasirox: Results from the EPIC Clinical Trial. Anemia 2012;2012:297641.

Porter J and Hershko C. The properties of Clinically useful Iron Chelators Iron Phyisology and Pathophysiology in Humans , Editors Anderson HJ and McLaren GD. Humana Press (Book). 2012;Chapter 28:591-630.

Porter J, Galanello R, Saglio G, et al. Relative response of patients with myelodysplastic syndromes and other transfusion-dependent anaemias to deferasirox (ICL670): a 1-yr prospective study. Eur J Haematol 2008;80:168-76.

Porter JB, Tanner MA, Pennell DJ, et al. Improved Myocardial T2* in Transfusion Dependent Anemias Receiving ICL670 (Deferasirox) (abstract). Blood 2005a;106:3600.

Porter JB, Abeysinghe RD, Marshall L, et al. Kinetics of removal and reappearance of non-transferrin-bound plasma iron with deferoxamine therapy. Blood 1996;88:705-13.

Porter JB and Davis BA. Monitoring chelation therapy to achieve optimal outcome in the treatment of thalassaemia. Best Pract Res Clin Haematol 2002;15:329-68.

Porter JB, Elalfy MS, Taher AT, et al. Efficacy and safety of deferasirox at low and high iron burdens: results from the EPIC magnetic resonance imaging substudy. Annals of hematology 2013a;92:211-9.

Porter JB, Faherty A, Stallibrass L, et al. A trial to investigate the relationship between DFO pharmacokinetics and metabolism and DFO-related toxicity. Ann N Y Acad Sci 1998;850:483-7.

Porter JB, Jaswon MS, Huehns ER, et al. Desferrioxamine ototoxicity: evaluation of risk factors in thalassaemic patients and guidelines for safe dosage. Br J Haematol 1989;73:403-9.

Porter JB, Lin KH, Beris P, et al. Response of iron overload to deferasirox in rare transfusion-dependent anaemias: equivalent effects on serum ferritin and labile plasma iron for haemolytic or production anaemias. Eur J Haematol 2011;87:338-48.

Porter JB, Rafique R, Srichairatanakool S, et al. Recent insights into interactions of deferoxamine with cellular and plasma iron pools: Implications for clinical use. Annals of the New York Academy of Sciences 2005b;1054:155-68.

Porter JB and Shah FT. Iron overload in thalassemia and related conditions: therapeutic goals and assessment of response to chelation therapies. Hematol Oncol Clin North Am 2010;24:1109-30.

Porter JB, Wood J, Olivieri N, et al. Treatment of heart failure in adults with thalassemia major: response in patients randomised to deferoxamine with or without deferiprone. Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance 2013b;15:38.

Rheault MN, Bechtel H, Neglia JP, et al. Reversible Fanconi syndrome in a pediatric patient on deferasirox. Pediatr Blood Cancer 2011;56:674-6.

Sharma R, Anand R, Chandra J, et al. Distal ulnar changes in children with thalassemia and deferiprone related arthropathy. Pediatr Blood Cancer 2013;

Singh S, Hider RC and Porter JB. A direct method for quantification of non-transferrin-bound iron. Anal Biochem 1990;186:320-3.

St Pierre TG, Clark PR, Chua-anusorn W, et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. Blood 2005;105:855-61.

Storey P, Thompson AA, Carqueville CL, et al. R2* imaging of transfusional iron burden at 3T and comparison with 1.5T. J Magn Reson Imaging 2007;25:540-7.

Taher A, El-Beshlawy A, Elalfy MS, et al. Efficacy and safety of deferasirox, an oral iron chelator, in heavily iron-overloaded patients with beta-thalassaemia: the ESCALATOR study. Eur J Haematol 2009;

Taher AT, Porter JB, Viprakasit V, et al. Deferasirox effectively reduces iron overload in non-transfusion-dependent thalassemia (NTDT) patients: 1-year extension results from the THALASSA study. Annals of hematology 2013;92:1485-93.

Tanner MA, Galanello R, Dessi C, et al. A randomized, placebo-controlled, double-blind trial of the effect of combined therapy with deferoxamine and deferiprone on myocardial iron in thalassemia major using cardiovascular magnetic resonance. Circulation 2007;115:1876-84.

Tanner MA, Galanello R, Dessi C, et al. Myocardial iron loading in patients with thalassemia major on deferoxamine chelation. J Cardiovasc Magn Reson 2006;8:543-7.

Telfer P. Update on survival in thalassemia major. Hemoglobin 2009;33 Suppl 1:S76-80.

Telfer P, Coen PG, Christou S, et al. Survival of medically treated thalassemia patients in Cyprus. Trends and risk factors over the period 1980-2004. Haematologica 2006;91:1187-92.

Thomas A, Garbowski M, Ang A, et al. A Decade Follow-up of a Thalassemia Major (TM) Cohort Monitored by Cardiac Magnetic Resonance Imaging (CMR): Significant Reduction In Patients with Cardiac Iron and In Total Mortality. Blood 2010;116:Abstract 1011.

Tondury P, Zimmermann A, Nielsen P, et al. Liver iron and fibrosis during long-term treatment with deferiprone in Swiss thalassaemic patients. British Journal of Haematology 1998;101:413-5.

Traynor K. Deferiprone approved for iron overload. Am J Health Syst Pharm 2011;68:2106.

Tsou AY, Friedman LS, Wilson RB, et al. Pharmacotherapy for Friedreich ataxia. CNS Drugs 2009;23:213-23.

Vichinsky M, Bernaudin, F, Forni GL, et al. Long-Term Safety and Efficacy of Deferasirox (Exjade®) In Transfused Patients with Sickle Cell Disease Treated for up to 5 Years (abstract). Blood 2010;116:845.

Vichinsky E. Clinical application of deferasirox: practical patient management. Am J Hematol 2008;83:398-402.

Villeneuve JP, Bilodeau M, Lepage R, et al. Variability in hepatic iron concentration measurement from needle-biopsy specimens. Journal of Hepatology 1996;25:172-7.

Viprakasit V, Ibrahim H, Ha SY, et al. Clinical efficacy and safety evaluation of tailoring iron chelation practice in thalassaemia patients from Asia-Pacific: a subanalysis of the EPIC study of deferasirox. Int J Hematol 2011;93:319-28.

Viprakasit V, Nuchprayoon I, Chuansumrit A, et al. Deferiprone (GPO-L-ONE([R])) monotherapy reduces iron overload in transfusion-dependent thalassemias: 1-year results from a multicenter prospective, single arm, open label, dose escalating phase III pediatric study (GPO-L-ONE; A001) from Thailand. American journal of hematology 2013;88:251-60.

Vlachodimitropoulou E, Garbowski M and Porter. JB. Modelling Combination Chelation Regimes to Optimize Cellular Iron Removal and explore mechanisms of enhanced chelation. Blood 2013;Abstract 2200.

Voskaridou E, Christoulas D and Terpos E. Successful chelation therapy with the combination of deferasirox and deferiprone in a patient with thalassaemia major and persisting severe iron overload after single-agent chelation therapies. British journal of haematology 2011;154:654-6.

Waldmeier F, Bruin GJ, Glaenzel U, et al. Pharmacokinetics, metabolism, and disposition of deferasirox in beta-thalassemic patients with transfusion-dependent iron overload who are at pharmacokinetic steady state. Drug Metab Dispos 2010;38:808-16.

Wanless IR, Sweeney G, Dhillon AP, et al. Lack of progressive hepatic fibrosis during long-term therapy with deferiprone in subjects with transfusion-dependent beta-thalassemia. Blood 2002;100:1566-9.

Wei HY, Yang CP, Cheng CH, et al. Fanconi syndrome in a patient with beta-thalassemia major after using deferasirox for 27 months. Transfusion 2011;51:949-54.

Wolfe L, Olivieri N, Sallan D, et al. Prevention of cardiac disease by subcutaneous deferoxamine in patients with thalassemia major. N Engl J Med 1985;312:1600-3.

Wood JC. Diagnosis and management of transfusion iron overload: the role of imaging. Am J Hematol 2007;82:1132-5.

Wood JC and Ghugre N. Magnetic resonance imaging assessment of excess iron in thalassemia, sickle cell disease and other iron overload diseases. Hemoglobin 2008;32:85-96.

Wood JC, Glynos T, Thompson A, et al. Relationship between labile plasma iron, liver iron concentration and cardiac response in a deferasirox monotherapy trial. Haematologica 2011;96:1055-8.

Worwood M, Cragg SJ, Jacobs A, et al. Binding of serum ferritin to concanavalin A: patients with homozygous beta thalassaemia and transfusional iron overload. Br J Haematol 1980;46:409-16.

Yadav SK, Gupta V, El Kohly A, et al. Perforated duodenal ulcer: a rare complication of deferasirox in children. Indian J Pharmacol 2013;45:293-4.

Yarali N, Fisgin T, Duru F, et al. Subcutaneous bolus injection of deferoxamine is an alternative method to subcutaneous continuous infusion. J Pediatr Hematol Oncol 2006;28:11-6.

Yesim Aydinok P, Patricia Evans, Dr.2, Aysen Terzi, PhD1,*, Nurten Cetiner, PhD1,* and John B. Porter. Randomised Prospective Evaluation of Iron Balance, Chelation Efficiency, Urine Excretion and NTBI Progression with Deferiprone (DFP) or Deferoxamine (DFO) Monotherapy or with Combined DFP Plus DFO (abstract). Blood 2005;106:269.

Zanninelli G, Breuer W and Cabantchik ZI. Daily labile plasma iron as an indicator of chelator activity in Thalassaemia major patients. Br J Haematol 2009;147:744-51.