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Comments and Critique

PML/RARα + ATRA = CR
A Comment on Acute Promyelocytic Leukaemia: Molecular Pathology and Treatment

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THE CHARACTERISATION of recurrent chromosomal translocations of the acute leukaemias has proved to be a happy hunting-ground for molecular biologists. Improvements in positional cloning techniques have allowed the rapid analysis of all the common breakpoints and those involved in the acute myeloid leukaemias (AML) are summarised in Table 1.

A common biochemical result is the production of novel "fusion" proteins, derived from the juxtaposition of genes which are normally separated in the genome [1]. In AML, individual fusion proteins are specific for a given morphological subtype, and are thought to be of central importance in the pathogenesis of the disease. Since these fusion proteins are not found in normal cells, they are good targets for monitoring response to therapy, either by specific antibodies or more routinely by RT-PCR (reverse transcriptase-polymerase chain reaction). This latter approach can be used routinely in a significant proportion of AML for which there were previously no good clonal markers. An objective assessment of the quality of the remission can now be made and should be mandatory in patients undergoing any form of bone marrow transplantation procedure.

The more tantalising prospect is to use these fusion proteins (or their mRNA or DNA) as targets for tumour-specific therapy. As can be seen from Table 1, the majority of these proteins are transcription factors of unknown function, which are expressed at relatively low levels within the nucleus of the cell. Targeting for *in vivo* therapy will, therefore, depend on the further development of new technologies including antisense oligonucleotides, specific RNA catalytic molecules (ribozymes) or immunotherapy strategies directed to intra-cellular antigens. Identification of the normal proteins which interact with the fusion proteins to produce the neoplastic phenotype may also result in other therapeutic options.

However, in one form of AML, acute promyelocytic leukaemia (APL), tumour-specific therapy has already arrived without any technological revolutions: all-*trans*-retinoic acid (ATRA), an orphan drug, is able to induce complete remission (CR) in virtually all patients with the t(15;17)(q24;q21) translocation (reviewed in [4, 5]). It had been known for several years that a variety of agents, including both *cis*- and *trans*-retinoic acids, could induce terminal differentiation of AML cells and cell lines *in vitro*, but it was Zhen-Yi Wang and colleagues in Shanghai who were the first to demonstrate that ATRA consistently induced CR in patients with APL. ATRA is ineffective against all AML which lack the t(15;17)(q24;q21), including the rare forms of APL with t(11;17)(q23;q21). *Cis*-retinoic acid lacks any consistent activity *in vivo*. CR is attained by terminal differentiation of the APL cells and, therefore, occurs without any intervening period of severa marrow aplasia, without exacerbating the APL-associated coagulopathy, and is generally well tolerated.

In itself, this is an intriguing observation, but is made doubly so by the fact that the gene disrupted at 17q21 in all forms of APL is the retinoic acid receptor-α (RARα) gene. In the great majority of APL, the RARα gene on chromosome 17 becomes fused with the ubiquitously expressed promyelocytic leukaemia (PML) gene on chromosome 15. More rarely, the RARα gene becomes fused with the promyelocytic leukaemia zinc finger gene on chromosome 11q23.1 [6]. Both the mode of action of ATRA and any effects it has on the various PML/RARα fusion products seen in APL with the t(15;17)(q24;q21) remain obscure.

So what's the catch? The major problem is that the ATRA-induced CRs are of brief duration, usually only a few months, and therefore conventional chemotherapy needs to be given immediately after ATRA. Resistance to ATRA is exceptional in *de novo* APL cases, but is seen frequently at relapse, apparently due to lack of bioavailability of the drug. Another problem, in the literature at least, has been how to best define APL. APL should be relatively straightforward to diagnose, with well-described cytological features, a characteristic immunophenotype ($CD13^+$, $CD33^+$, $CD34^-$, $HLA\text{-}DR^-$, $CD11b^-$ with the cytological variant form of APL also expressing CD2, another possible target for selective therapy), and all should exhibit rearrangement of 17q21 cytogenetically. However, the clinical problem is whether to give ATRA to patients in whom the t(15;17)(q24;q21) cannot be demonstrated cytogenetically. Up to 15% of cases have been reported to have normal karyotypes even when using the correct culturing conditions. Cases with normal chromosomes 15 and 17 may, nevertheless, have a

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Table 1. Molecular characterisation of chromosomal translocations in acute myeloid leukaemias

Disease subtype	% of all AML	Chromosomal translocation	Fusion product	Reference
1. Differentiated myeloid (FAB AML M2)	5–20	t(8;21)(q22;q22)	CBFα (AMLI)-ETO	2
2. Myelomonocytic with eosinophilia (M4Eo)	8	inv (16)(p13;p32)	CBFβ-MYH 11	3
3. Promyelocytic	10	t(15;17)(q24;q21)	PML-RARα	4, 5
(M3/M3v)	Rare	t(11;17)(q23.1;q21)	PLZF-RARα	6
4. AML with basophilia	1–2	t(6;9)(p23;q34)	DEK-CAN	7

Fusion products deriving from translocation in AML generally represent the fusion of two transcription factors. Exceptions are MYH11 (smooth muscle myosin heavy chain) and RARα (retinoic acid receptor-α). PML, promyelocytic leukaemia gene; PLZF, promyelocytic zinc finger gene. Note that the frequency of the t(8;21) varies with geographical location.

PML/RARα fusion, a condition analogous to chronic myeloid leukaemias which lack cytogenetically the t(9;22)(q34;q11), but have molecular evidence for BCR-ABL fusion [8]. Since clinical efficacy of ATRA depends on the expression of the PML/RARα gene, evidence for this fusion product should be sought in all cases where such therapy is being considered.

The efficacy of ATRA in APL is a most exciting development. Much remains to be learnt, both clinically and scientifically. Might other forms of retinoic acid produce more durable CRs? Most importantly, do patients treated with ATRA fare as well as those treated with chemotherapy alone? (APL is one of the "best" subgroups of AML with regard to outcome.) Finally, given the experience in APL, do other translocations in other malignancies involve other RAR genes of which there are several, and if so, can the same approach to therapy be adopted?

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Serum Neurone-specific Enolase and Other Neuroendocrine Markers in Lung Cancer

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SMALL CELL lung cancer (SCLC) can be distinguished from other types of lung cancer by its morphology, biological behaviour and chemosensitivity. Neuroendocrine differentiation is consistent, although not a specific feature of SCLC, and different patterns of neuroendocrine expression can be found in 'classic' and 'variant' subtypes of SCLC lines [1]. This raises the possibility that neuroendocrine markers might identify biological properties of SCLC which could provide useful clinical information.

Neurone-specific enolase (NSE), a neuronal form of the glycolytic enzyme enolase consistently found in SCLC lines, is a secreted biochemical marker of neuroendocrine tumours and SCLC [2]. The neuronal form of the enzyme contains either alpha-gamma or gamma-gamma subunits, and raised levels of NSE are found in the serum of 66–81% of patients with SCLC [3–5]. The detection of raised levels of NSE in serum correlates with its presence in tissue [6]. Elevated levels of other neuroendo-

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