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REVIEW

CYTOGENETICS AND THE MOLECULAR APPROACH TO THE DIAGNOSIS AND MANAGEMENT OF ACUTE LEUKEMIA IN ADULTS

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ABSTRACT. ACUTE LEUKEMIA is a heterogeneous hematological malignancy that is well characterised on a molecular level. This study presents the management of 70 consecutive patients with adult acute leukemia presenting to a single institution. Cytogenetic analysis was performed on all patients and was used to risk-stratify. The majority of patients were then treated as part of larger multi-institution trials to improve the outcome of adult acute leukemia. The results of these trials are now known, and will be presented. Molecular characterizations have made significant contributions to the diagnosis of acute leukemia in terms of cytogenetic classification and risk factor stratification. Subsequently, molecular targeted therapy has led to the development of new potential avenues for therapy, which have already shown activity in acute leukemia. Future treatment approaches may involve molecular based therapy in combination with standard cytotoxic chemotherapy.

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1. INTRODUCTION

Acute leukemia is a hematological malignancy affecting approximately 13 adults per 100,000 per year in South Australia [1]. Acute myeloid leukemia (AML) is predominantly a disease of adults, with increasing incidence with older age. Only 4% of sufferers are under 15 years of age. By contrast, 54% of patients with acute lymphoblastic leukemia (ALL) are under 15 years of age. Between 1977 and 1995, the overall 5-year survival for ALL was 52% in contrast to AML for which it was 10%. Impressive improvements in outcome for some patients have been demonstrated. Early treatment advances have mainly been in the form of treatment intensification, but intensification has limitations even with the use of hematopoietic stem cell rescue. The improved survival for ALL has been predominantly in children. These improvements have been attributed to the intensification of chemotherapy. For older patients, intensification has been significantly limited by toxicity. Future advances may therefore be based upon molecular understanding of the pathogenesis of acute leukemia, and depend upon the molecular characterization and therapeutic targeting of leukemia-specific molecules. By the late 1990s, molecular-based targeted therapy had already become part of standard therapy, manifested in the molecular diagnosis of promyelocytic leukemia (APML) based upon detection of the translocation t(15;17), and subsequent treatment with retinoic acid. The discovery of internal tandem duplication (ITD), and development of ITD inhibitors, ABL inhibitors, vascular endothelial growth factor (VEGF) inhibitors, histone deacetylase (HDAC) inhibitors, DNA methylation inhibitors, and conjugated anti-CD33 monoclonal antibodies have been added to the list of molecular targeted therapies.

2. RISK FACTOR CLASSIFICATION OF ACUTE LEUKEMIA [2-15]

To investigate the heterogeneity of a cohort of patients presenting to a single institution, 70 consecutive patients diagnosed with acute leukemia by bone marrow biopsy at the Royal Adelaide Hospital (RAH) between October 1996 and October 1998 were classified according to bone marrow blast morphology, immunophenotype, and cytogenetics. Bone marrow morphology was classified according to the French-American-British (FAB) criteria (FIG. 1A) [12,13]. Cytogenetic analysis was performed using G-banding of short-term unstimulated cultures. Clonal chromosomal abnormalities were found in 46 out of 69 (67%) evaluable patients (FIG. 1B). In one patient with multi-organ failure there were insufficient metaphases cultured to determine the karyotype. Risk factor assignment into good, intermediate, or poor risk according to cytogenetics, the presence of an antecedent haematological disorder preceding the diagnosis, high white cell count ($>100 \times 10^9$/L for AML or $>30 \times 10^9$/L for ALL), blasts with undifferentiated morphology, and poor performance status due to multi-organ failure were made at presentation, and are outlined in TABLE 1. In many cases amongst patients with a poor prognosis, more than one poor prognostic feature was present.

Overall, 13% of patients were good risk, 36% intermediate risk, and 51% poor risk. Amongst patients with AML, 14% were good risk, 40% intermediate risk, and 46% poor risk compared to patients with ALL for which the respective figures were 9%, 17% and 75%. With increasing age, there were fewer good risk patients and more poor risk patients (FIG. 2). For patients under 50, the distribution of good, intermediate and poor risk was 26%, 41%, and 33%. For patients 50 and over, the respective distribution was 5%, 33% and 63% ($P = 0.011$).

Multiple poor risk features were often found together. Patients with complex cytogenetics were all between the ages of 36 and 78, and the average age was 64. Of seven karyotypes with complex cytogenetics, three included either 5q-, 7q-, 5q-, 7-, or t(9;22). One of these was from a patient with a documented antecedent haematological disorder (myelodysplasia). A further complex karyotype occurred following prior chemotherapy for Ew-

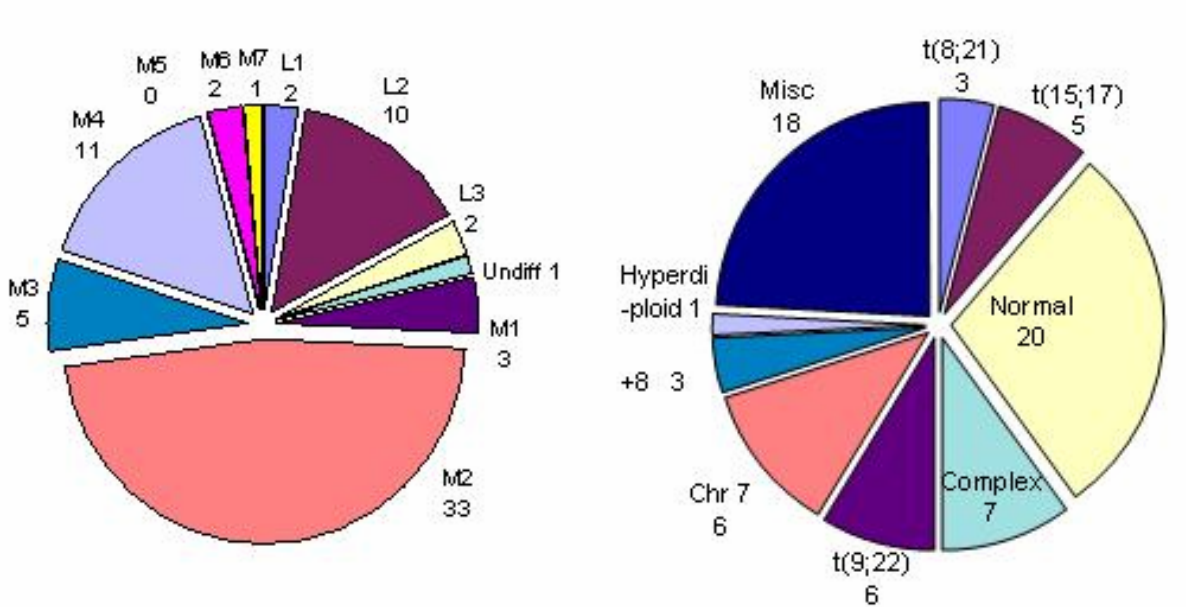

FIGURE 1. CYTOGENETIC PROFILE OF ADULT ACUTE LEUKEMIA. Cytogenetic analysis was successful in 69/70 patients. In one patient who presented with sepsis and died in intensive care, there were insufficient metaphases for analysis.

TABLE 1. RISK ASSIGNMENT.

GOOD RISK:	
t(15;17)	5
t(8;21)	3
Hyperdiploid ALL	1
TOTAL NO. OF PATIENTS	**9**
INTERMEDIATE RISK:	
Normal karyotype	19
+8	2
Nonspecific karyotype	3
t(8;14)	1
TOTAL NO. OF PATIENTS	**25**
POOR RISK:	
Complex karyotype	7
Abnormal chromosome 7	6
t(9;22)	6
AHD*	5
t(6;9), inv(3), t(9;11), t(11;19)	6
High WCC**	4
Sepsis	1
Undifferentiated morphology	1
TOTAL NO. OF PATIENTS	**36**

* Antecedent haematological disorder.
** ~30 x 10^9/L in ALL, 100 x 10^9/L AML.

ing's sarcoma. This patient was the youngest with a complex karyotype at age 36. Six karyotypes (five ALL, one AML) were positive for t(9;22). One of these also had complex cytogenetics, two were associated with a white cell count (WCC) > 100 x 10^9/L, and one occurred following prior chemotherapy (cyclophosphamide, methotrexate and 5-fluorouracil) for breast cancer. Karyotypes positive for t(9;22) were all from patients aged between 29 and 55, with an average age of 44. This younger age for patients with t(9;22) positive acute leukemia compares with the Groupe Francais de Cytogenetique Hematologique of 443 adult patients, where the peak age of t(9;22) was in the 40 to 50 year old group [11].

The 12 patients with ALL in this cohort were on average younger than the patients with AML (mean age 44, range 18-72). Eight patients were under 50 years of age, 4 were over 50. Overall, there were proportionately more patients with poor risk disease. There was 1 patient with good risk, 2 with intermediate risk, and 9 with poor risk disease.

TABLE 2. SUMMARY OF VARIOUS TREATMENTS.

TREATMENT	NO. OF PATIENTS
ICE[1]	18
Mitoxantrone / Ara-C[2]	10
DAV[3]	6
ATRA[4]	4
LALA[5]	6
Hoelzer[5]	3
LMB 199328[6]	1
DVP[7]	2
Palliative	20

1. AML < age 60.
2. AML > age 60.
3. MGDF study.
4. Promyelocytic leukemia.
5. Adult ALL intensive treatment.
6. L3, t(8;14) positive.
7. ALL "gentle" therapy.

3. TREATMENT

The treatment given could be regarded as standard therapy for the late 1990s (TABLE 2).

3.1. AML

Patients under age 60 were enrolled in the Australasian Leukaemia and Lymphoma Group (ALLG) AML trial number 7 (M7) protocol [16]. M7 was a prospective randomized trial evaluating sequential courses of chemotherapy containing high-dose cytarabine in both induction and consolidation therapy for AML, in addition to the use of G-CSF to reduce toxicity. The "ICE" protocol (idarubicin 9 g/m^2 3 days, cytarabine 3 g/m^2 bd alternate daily for 8 doses, etoposide 75 mg/m^2 daily 7 days) was used for induction. Patients in remission were then randomized to either a further identical cycle of ICE or 2 courses of cytarabine 100 mg/m^2 daily in combination with idarubicin and etoposide (IcE). Patients over age 60 were included in the ALLG study on the role of mitoxantrone and intermediate dose cytarabine 3 g/m^2 in AML for patients over 60 [17]. A separate study to investigate the role of megakaryocyte growth and development factor (MGDF) used the "DAV" protocol (daunorubicin 45 g/m^2 daily 3 days, cytarabine 100 mg/m^2 daily for 7 days, etoposide 75 mg/m^2 for 7 days). In both cases, only two courses of consolidation were required. Patients over age 60 were treated with mitoxantrone 10 mg/m^2 for 3 days and cytarabine 500 mg/m^2 bd for 3 days as induction therapy. Maintenance therapy was not used for AML. For APML, retinoic acid induction therapy was used in combination with idarubicin. Patients entering remission were then given two courses of consolidation doses of idarubicin, cytarabine and etoposide.

3.2. ALL

The Hoelzer 88 protocol was used initially, but was later replaced by the French LALA94 protocol [18,19]. In general, these intensive ALL regimens involve multiple drugs given for up to 2 years as induction, consolidation, intensification, and maintenance. CNS prophylaxis is also required in the form of intrathecal chemotherapy and cranial irradiation. For patients with a poor performance status, or elderly patients, more gentle induction with DVP (daunorubicin, vincristine and prednisolone) was used. For L3 ALL, the LMB 199328 protocol was used as intensive therapy for the treatment of L3 ALL [20,21].

3.3. ALLOGENEIC TRANSPLANTATION IN AML AND ALL

For eligible patients under 50 years of age, allogeneic transplantation was offered in first complete remission (CR) for intermediate or high-risk patients. For all other patients, allogeneic transplantation was offered in second CR. Autologous transplantation was given to one standard risk patient in second CR and one high-risk patient in first CR. Of the 50 patients treated, 15 underwent high dose therapy with allogeneic or autologous transplantation.

3.4. PALLIATIVE CARE

Twenty patients elected for supportive care only. All were diagnosed with AML, and repre-

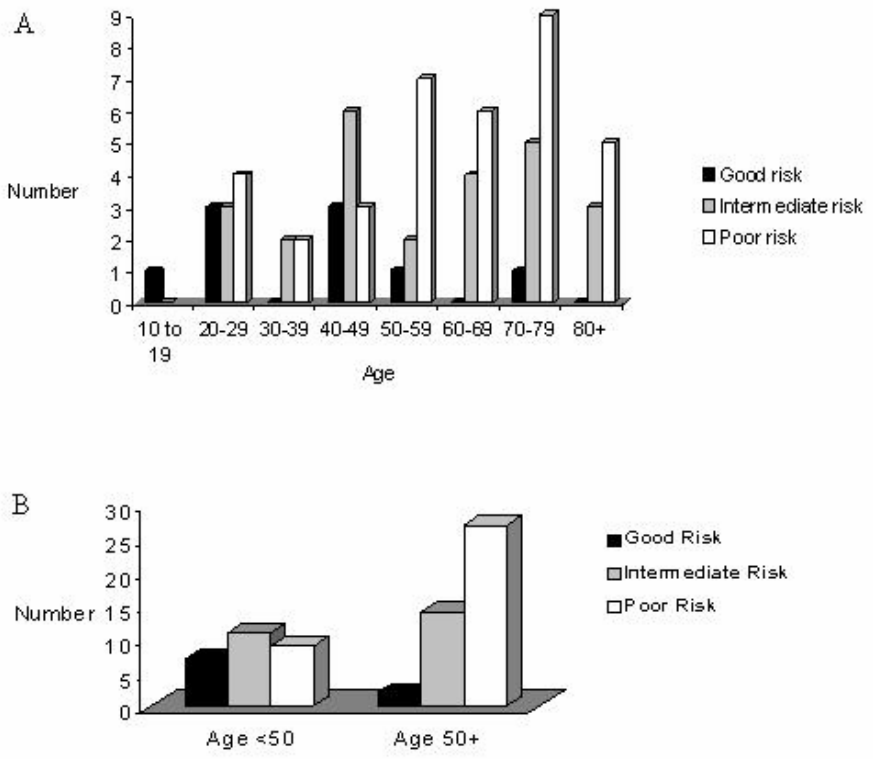

FIGURE 2. CLASSIFICATION OF ACUTE LEUKEMIA ACCORDING TO RISK FACTORS. A, B. There was an increase in poor risk features with increasing age. B. For patients under 50, the distribution of good, intermediate and poor risk was 26%, 41%, and 33% respectively. For patients 50 and over, the respective distribution was 5%, 33% and 63% (P = 0.011).

sented 34% of patients diagnosed with AML. The mean age was 77, (range 58 to 87). Five were intermediate risk, 14 were poor risk, and 1 good risk. The youngest patient was 59 and had good risk but not treated due to dementia. The others were all over age 60, with 5 intermediate risk and 14 poor risk.

4. RESPONSE TO THERAPY

4.1. INDUCTION THERAPY AML

Fifty-eight patients were diagnosed with AML (mean age 58, range 21-87). One good risk patient aged 59 elected not to undergo chemotherapy because of Huntington's Chorea with early onset dementia. Of the 38 patients treated, the mean age was 50 (range 21-76). Twenty-eight achieved CR (74%). Of 8 patients with good risk cytogenetics (mean age 37, range 23-73), all 7 patients (100%) who were treated achieved CR. Twenty three patients had intermediate risk (mean age 56, range 21-87). Eighteen of these 23 patients (78%) were treated (mean age 50, range 21-76). Thirteen out of 18 (72%) achieved CR. Twenty seven patients had poor risk disease (mean age 65, range 27-86). 13 (48%) were treated (mean age 54, range 27-74). Eight out of 13 (62%) achieved CR. Patient age at presentation was a significant independent risk factor for achieving CR. Nineteen out of 19 patients aged less than 50 were treated. Seventeen of 19 treated patients (89%) achieved CR. The one year survival was 63% overall, and 71% if CR was achieved. For patients over age 50, only 19 of 39 or 49% of patients elected for treatment. 11 of 19 patients (58%) achieved CR. The one year survival was 11% overall, but 18% if CR achieved. One patient with t(15;17) was aged 73 at diagnosis, and was the only good risk patient with AML or ALL

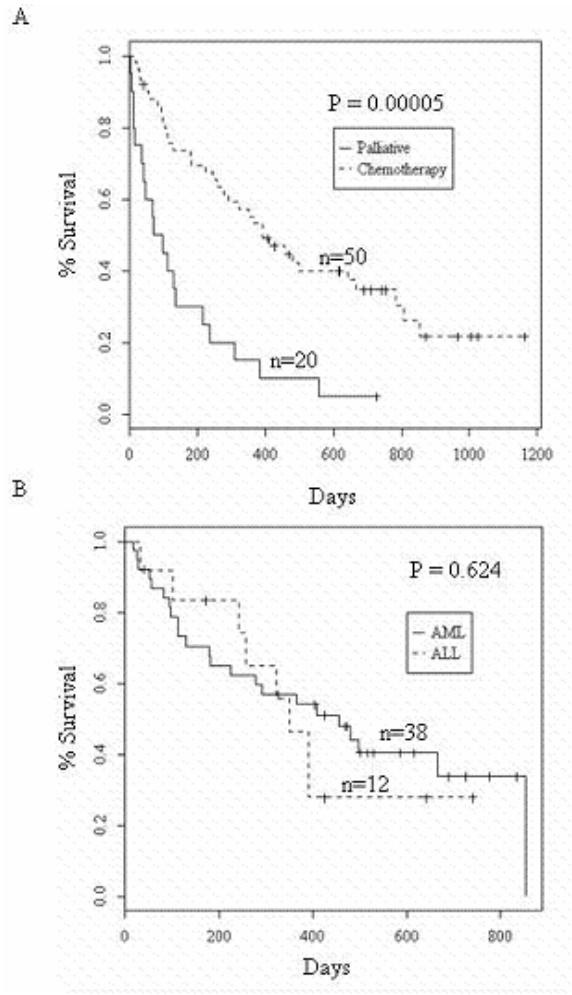

FIGURE 3. A. SURVIVAL IN DAYS OF ALL PATIENTS. Palliative mean = 160, median = 84. Patients treated with chemotherapy mean = 525, median = 392. B. Survival in days of all patients treated with chemotherapy. AML mean = 538, median = 455. ALL mean = 435, median = 371.

over 50 years of age. This patient achieved CR after treatment with retinoic acid therapy, was lost to follow up but died of undetermined causes 28 months after diagnosis.

4.2. INDUCTION THERAPY ALL

The 12 patients with ALL in this cohort were on average younger than the patients with AML (mean age 44, range 18-72). Eight patients were under 50 years of age, 4 were over 50. Ten patients (83%) achieved CR. All 12 patients were treated with the various protocols outlined in table 2. Ten patients (83%) achieved CR. One patient, who had leukemia secondary to prior chemotherapy, died during induction therapy from complications of marrow aplasia. The other failure to achieve CR was in a 70 year old with t(8;14) positive L3 ALL, who did not tolerate high dose methotrexate therapy.

4.3. BONE MARROW TRANSPLANTATION

Fifteen patients underwent bone marrow transplantation (mean age 37, range 23-61). Thirteen were allogeneic transplants including 3 from matched unrelated donors. Five allogeneic transplants were for relapsed disease in second remission (3 standard risk, 2 good risk, all AML). Seven allogeneic transplants were performed in first remission, including 5 high-risk and 2 intermediate risk patients. One transplantation was performed for resistant disease (Philadelphia positive AML). Two autologous transplants were performed, one for secondary acute leukemia, the other for relapsed disease in a patient without an allogeneic donor. Eleven patients died between 4 and 27 months from diagnosis (average 16 months). Six deaths were from graft versus host disease, 2 deaths from disease relapse, 1 from veno-occlusive disease, 1 autologous transplantation and 1 MUD transplantation died from early complications of the procedure.

4.4. SURVIVAL (FIG. 3-5)

For all patients who were treated, both AML and ALL, log-rank analysis of Kaplan-Meier survival curves was used to compare the outcome between AML and ALL, and between different risk groups for all ages as well as below and above age 50 (FIG. 3–5). A Cox proportional hazards model was fitted to the data indicator variables. These models showed that the term corresponding to risk was not needed in the model in the presence of age, while age may add something to the model that contains risk. In the model with only age, the

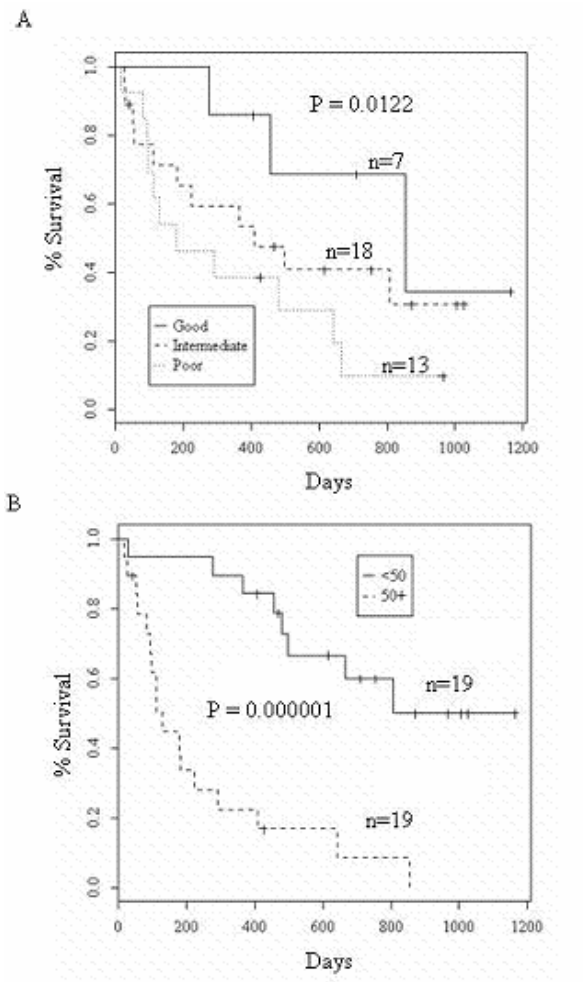

FIGURE 4. A. SURVIVAL IN DAYS OF ALL PATIENTS WITH AML TREATED WITH CHEMOTHERAPY ACCORDING TO RISK. AML good risk mean = 810, median = 854. AML intermediate risk mean = 514, median = 409. AML poor risk mean = 341, median = 179. B. Survival in days of all patients with AML aged <50 treated with chemotherapy versus all patients aged >50 treated with chemotherapy. AML age <50 mean = 825, median = 807. Age >50 mean = 240, median = 129.

hazard ratio for a patient aged 50+ relative to <50, was about 5. However, there was only one patient treated over 50 with good risk disease. Although this patient was the longest survivor in this age group at 23 months, statistical analysis of an age effect on patients with good risk was not meaningful due to the small numbers involved. Similarly, there was only one good-risk ALL patient in this present cohort of adult patients. No statistically significant difference was seen between patients with ALL in different risk groups or age categories. The average survival for the 20 patients treated with supportive therapy only was 3 months, with 5 patients surviving beyond 6 months.

5. DISCUSSION

5.1. AML CYTOGENETICS AND RISK ASSIGNMENT

Cytogenetic analysis was performed in all patients, and was successful in 99%. Patients were grouped into either good-risk (13% of patients), intermediate risk (36%) or poor risk (51%) categories. For all patients under age 50, the distributions were 26%, 41%, and 33% respectively. When considering only those patients under age 50 with AML, the distribution was 32% good risk, 53% intermediate, and 16% poor risk. These results are similar to those reported by the UK AML MRC 10 study for patients under age 55 [22], as well as the South West Oncology Group (SWOG) review of cytogenetics in elderly AML [5] and the University of Maryland Cancer Centre in 1989 [23]. Furthermore, both the Maryland and the Adelaide studies demonstrated a progressive deterioration in prognosis for each decade increase in age.

5.2. DOSE INTENSIFICATION AND OUTCOME

In 1984, Schiffer et al defined three statistically significant clusters of patients [14]. Those with 16q22 abnormalities had the best prognosis, whereas patients with t(15;17) or t(8;21) had an intermediate prognosis, and patients with abnormalities of chromosomes 5, 7, 11q23 or chromosome 8 had a poor prognosis. In 1988, the MD Anderson reported a high remission rate (>90%) for t(8;21) and inv16, but the remission duration for patients with t(8;21) tended to be similar to patients with normal cytogenetics and an intermediate prognosis [9]. Hence t(8;21) could

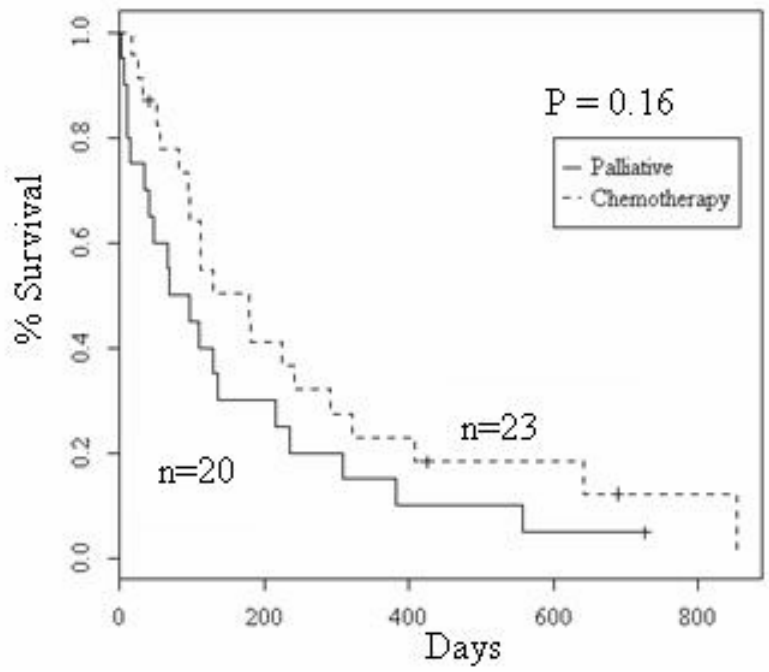

FIGURE 5. OUTCOME OF ALL PATIENTS OVER AGE 50 TREATED WITH CHEMOTHERAPY COMPARED TO PALLIATIVE SUPPORTIVE CARE ONLY. Mean = 139, median = 84. Chemotherapy mean = 259, median = 179.

have been regarded as a good risk at presentation, but intermediate risk when considering remission duration.

The combination of an anthracycline and cytarabine has been standard therapy for AML for many years. During the 1980s, combination chemotherapy with daunorubicin, low dose cytarabine, and thioguanine was widely used therapy for AML. Between 1984 and 1990, the MRC AML 9 trial recruited 972 patients to compare a total dose of 50 mg/m^2 with 150 mg/m^2 of daunorubicin and total doses of 1 g/m^2 versus 2 g/m^2 each for cytarabine and thioguanine as induction chemotherapy for AML [24]. The 5-year relapse free survival was 28% versus 23%, and survival was 23% versus 18% in favour of the more intense doses.

Between 1984 and 1991, the Australian Leukaemia Study Group (ALSG) randomised patients to receive standard dose cytarabine and daunorubicin (7-3), 7-3 plus etoposide (7-3-7) or 7-3 plus high-dose cytarabine (HIDAC-3-7) chemotherapy. An estimated 86% of 470 de novo patients with acute myeloid leukemia failed within 10 years of randomisation, as a result of death in induction (17%), failure to achieve CR (17%), relapse (44%), and death in CR (8%). Age, peripheral blast count and cytogenetics were significantly associated with disease-related failures. High-dose cytarabine in induction as well as allogeneic transplantation in first CR significantly decreased the disease-related failures. The impact of high-dose cytarabine did not depend on the cytogenetic risk group [25]. For patient under age 55, the addition of etoposide to daunorubicin and cytarabine did not significantly improve the CR rate, but improved median remission duration from 12 months to 27 months. Older patients experienced significantly more toxicity (grade 3 or 4 stomatitis) but no added benefit in outcome from the addition of etoposide [26].

During the 1990s, the ALLG compared induction with high dose versus lower dose continuous

infusion with cytarabine in combination with an anthracycline [27]. The CR rates were similar at 71% and 74% respectively. The 5-year survivals were also similar at 31% compared to 25%. The major differences in favour of high dose cytarabine were median remission duration of 45 months compared to 12 months, median disease free survival 22 months compared to 12 months, and 5-year disease free survival 49% compared to 24%. The magnitude of this improvement closely approximated the benefit seen in the Cancer and Leukemia Group B (CALGB) consolidation study [28]. In 1994, the CALGB reported a 4-year relapse free survival of 44% with high dose cytarabine versus 24% with low dose cytarabine for the treatment of AML [28]. The South West Oncology Group (SWOG) conducted a more complex study, which included an initial randomisation to high- or conventional-dose cytarabine [29]. Again, as in the ALLG study, there was no difference in CR rates between the 2 arms, but the high-dose arm was associated with improved relapse-free survival, suggesting that increased dose intensity of cytarabine in the initial treatment course resulted in greater kill of leukemic cells with improved long-term cure rates [29]. The SWOG study included a second randomisation for patients who responded to conventional-dose cytarabine induction therapy to then receive either 2 cycles of conventional-dose consolidation treatment or a single cycle of high-dose cytarabine. Patients who responded to high-dose cytarabine induction received a second cycle as consolidation treatment. This study was unable to confirm the improved relapse-free survival results of the CALGB study for the high-dose cytarabine consolidation arm. However, the small group of patients who received high-dose cytarabine both in induction and consolidation phases of treatment had the highest rate of relapse-free survival, although this did not reach statistical significance [29]. The CALGB has reported the outcome of t(8;21) patients assigned to one cycle of high-dose cytarabine was significantly inferior, with 62% of patients experiencing relapse with a median failure-free survival of 10.5 months, compared with the group of patients who received 3 or more cycles, in which only 19% experienced relapse, and failure-free survival was greater than 35 months. Furthermore, overall survival (OS) was also significantly compromised in patients assigned to one cycle of high-dose cytarabine, with 59% having died as a consequence of AML, compared with 24% of those who received 3 or more cycles of high-dose cytarabine [30]. However, for patients younger than 60 years with inv(16) or t(16;16) who had attained a complete remission on one of four consecutive clinical trials and were assigned to receive HDAC consolidation therapy, the 5-year OS was similar at 75% for the three to four cycles of HDAC group versus 70% for the one cycle of HDAC group [31], which may reflect a high success rate with stem-cell transplantation salvage treatment administered among patients in both treatment groups. Alternatively, blasts positive for inv(16)(p13q22) or t(16;16)(p13;q22) may have increased sensitivity to cytarabine. Based upon these results, the CALGB include three repetitive courses of HDAC consolidation for treatment of all t(8;21), inv(16), or t(16;16) patients in first CR.

Patients with t(15;17) were previously considered as having a good prognosis provided the patient survived the increased early mortality from haemorrhagic complications. In the late 1980s, Keating et al. [9] and Fenaux et al. [24] reported good remission rates were later complicated by high relapse rates for patients with t(15;17) and t(8;21). The rate of remission for t(15;17) was previously lower than for t(8;21) at 52%, but remission duration was comparable to patients with inv(16). Fenaux et al. [24] reported a high remission rate for patients with t(8;21) and t(15;17), but a median disease free survival of 12.5 months for t(15;17), and an actuarial disease free survival of 54% at 12 months for t(8;21). Thus, the good remission rates were later complicated by high relapse rates. Over subsequent years, however, the introduction of retinoic acid for APML leukemia and high dose cytarabine for other forms of AML appear to have improved that outlook [24,27,28,32-35]. During the 1990s, the introduction of retinoic acid therapy during induction has seen a dramatic reduction in early bleeding

complications for APML [33-35]. The addition of idarubicin to retinoic acid has reduced the complication of retinoic acid syndrome, and induction therapy for APML has been transformed from a dangerous procedure into a safe procedure, which can be managed almost entirely on an outpatient basis. Maintenance therapy with retinoic acid has been shown to be effective in reducing the relapse rate in APML Furthermore, patients who relapse, or are resistant to retinoic acid, may respond to arsenic [37-41]. Retinoic acid represents a turning point in the development of molecular target based therapies for acute leukemia, as discussed below.

The outcome of the ALLG AML trial M7 to assess sequential courses of chemotherapy containing high-dose cytarabine in both induction and consolidation therapy are now known [16]. Two hundred and ninety two AML patients aged 15 to 60 years were evaluated. Complete remission was achieved in 234 (80%) patients with ICE induction (idarubicin 9 g/m^2 3 days, cytarabine 3 g/m^2 bd alternate daily for 8 doses, etoposide 75 mg/m^2 daily 7 days). Two hundred and two patients in remission were then randomised to either a further identical cycle of ICE or 2 attenuated courses of cytarabine at 100 mg/m^2 daily in combination with idarubicin and etoposide (IcE). Although ICE consolidation was more toxic than IcE, the treatment-related death rate was not significantly different. There was no difference between the 2 consolidation arms for relapse-free survival at 3 years (49% for ICE versus 46% for IcE), survival following randomization (61% versus 62%), or the cumulative incidence of relapse (43% versus 51%), and there were no differences within cytogenetic groups. Although high-dose cytarabine induction resulted in high CR rates, further intensive consolidation treatment did not appear to confer additional benefit. Patients with normal cytogenetics gained a marginal benefit.

Maintenance chemotherapy versus intensified consolidation therapy for patients with AML has been shown to improve relapse free survival using 6-thioguanine, cytarabine, and daunorubicin (TAD) plus cytarabine and mitoxantrone (HAM; cytarabine 3 g/m^2 age <60 years or 1 g/m^2 age ≥60 years x 6) induction, TAD consolidation, and monthly modified TAD maintenance for 3 years, or TAD-HAM-TAD and one course of intensive consolidation with sequential HAM (S-HAM) with cytarabine 1 g/m^2 (age <60 years) or 0.5 g/m^2 (age ≥60 years) x 8 instead of maintenance, but OS was not significantly improved [42]. Patients with complex chromosomes treated with either TAD-TAD (thioguanine, daunorubicin, cytarabine) or TAD-HAM (high-dose cytarabine, mitoxantrone) have achieved a CR rate of 44%. Thirty-eight percent were non-responders and 18% experienced early death. The median OS was 8 months and the OS rate at 3 years was 6%, and the investigators concluded that de novo AML with complex cytogenetics remained a poor prognosis, even when patients were treated with intensive chemotherapy [43].

5.3. AML IN OLDER PATIENTS

The median age of AML is over 60 years [44]. Randomized studies have demonstrated a lack of survival benefit for older patients receiving intensive standard chemotherapy [26-28]. The results of the phase II ALLG clinical trial for fit elderly patients with de novo AML who received mitoxantrone and intermediate dose cytarabine (MIDAC) are now known. Less than half the patients achieved CR after induction and many of those in CR could not receive planned consolidation cycles. The median OS for all patients was 6.5 months and the median disease-free survival for those achieving CR was 8.3 months [17]. Similarly, a study from the Dana-Farber Cancer Institute demonstrated that cytarabine in combination with mitoxantrone was more toxic and no more effective than cytarabine alone, and higher-dose therapy had no benefit in the post-remission management of older patients with de novo AML [45]. Other investigators have attempted to improve outcome for older patients with AML by intensifying therapy. The MRC AML11 study randomized patients to 2 courses of

DAT (daunorubicin, cytarabine, and thioguanine) 3 + 10, ADE (daunorubicin, cytarabine, and etoposide) 10 + 3 + 5, or MAC (mitoxantrone-cytarabine). The remission rate in the DAT arm was significantly better than ADE (62% versus 50%) or MAC (62% versus 55%). However, there were no differences between the induction schedules with respect to OS at 5 years (12% versus 8% versus 10%) [46]. For APML in older patients, in a recent report from the European APL group, the CR rate was 86% in patients over age 60 as compared to 94.5% in younger patients (P = 0.0014), due to a higher incidence of early deaths in elderly patients. The 4-year survival of elderly patients was 57.8% as compared to 78% in younger adults (P = 0.0001). Therefore, APML in elderly patients was as sensitive to ATRA based regimen as in younger adults, but outcome was inferior mainly due to increased early deaths and to toxicity of consolidation treatment. Less intensive consolidation chemotherapy or possibly the use of arsenic may have more of a role to play in the treatment of APL in the elderly [47].

5.4. TRANSPLANTATION IN AML

The role of transplantation in AML in first remission has been well studied. Transplantation has been associated with severe morbidity and mortality. Knowing which patients were cured and which were destined to relapse was problematic, and randomized trials were therefore needed to determine whether transplantation was of benefit.

In 1998, the Eastern Cooperative Oncology Group (ECOG), the SWOG, and the CALGB reported an inter-group study of post-remission therapy for patients aged 15-55 in first CR. Patients with histocompatible siblings were assigned to allogeneic marrow transplantation, and the remaining patients were randomly assigned to high-dose cytarabine or autologous marrow transplantation [48]. No survival advantage was found for transplantation over intensive chemotherapy. Cytogenetics were reported only to illustrate comparability between groups, but no difference in outcome could be reported between different cytogenetic groups because of small numbers within the groups. In agreement, a study from the ECOG in 1992 failed to demonstrate a significant advantage for allogeneic transplantation for AML in first remission [49], and the Groupe Ouest Est Leucemies Aigues Myeloblastiques (GOELAM) study reported in 1997 also failed to show a benefit for transplantation in first remission [50].

In 1995 the European Organization for Research and Treatment of Cancer (EORTC) and the Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) Leukemia Cooperative Groups reported a trial comparing standard chemotherapy to autologous transplantation and allogeneic transplantation in first remission. Cytogenetic analysis was performed in only 44% of patients, whereas the final results were reported for all patients together [51]. There was an increased disease free survival in the transplantation groups, but OS was similar.

The MRC AML 10 study reported outcome according to cytogenetics of all 1,612 patients enrolled [22]. There was a strong correlation between cytogenetic risk factors with outcome. Of these patients, 381 were randomised to autologous transplantation versus chemotherapy. There was a statistically significant reduction in relapse rates for good and intermediate risk patients, and increased disease free survival for good risk patients treated with autologous transplantation. Overall survival was better if autologous transplantation was used as consolidation but this was not statistically significant.

In 2003, the European Organization for Research and Treatment of Cancer Leukemia Group and Gruppo Italiano Malattie Ematologiche dell' Adulto (EORTC-LG/GIMEMA) acute myeloid leukemia (AML)-10 trial, patients in first CR received a single intensive consolidation course. Subsequently, those patients younger than 46 years with an HLA-identical sibling donor were assigned to undergo allogeneic transplantation, and patients without such a donor were planned for autologous transplantation. Intention-to-treat analysis revealed that the 4-year survival rate was

58.3% versus 50.8% (P = 0.18). The disease-free survival (DFS) rates in patients with and without a sibling donor were similar in patients with good/intermediate risk but were 43.4% and 18.4%, respectively, in patients with bad/very bad risk cytogenetics. In younger patients (15-35 years), the difference was more pronounced [52]. In APML patients in second CR, autologous bone marrow transplantation with PML/RARα negative marrow cells is likely to result in prolonged clinical and molecular remissions [53].

Among 2,752 patients with acute leukemia who had recurrent leukemia after autograft in remission reported to the EBMT, 94 underwent allogeneic bone marrow transplantation and 74 received a second autograft. Although treatment related mortality at 2 years was 52% in recipients of matched allografts and 27% following a 2nd autograft, 2-year DFS was 27% and 35% in the two groups, respectively [54]. For these patients, novel therapies are required

5.5. ADULT ALL

For adult patients with ALL, the prognosis generally remains poor [15,55-57]. There has been improvement in the therapy of B-ALL and T-ALL with intensive therapy including intensification schedules and high dose methotrexate, but for patients achieving CR, long term DFS is still approximately 25-30% [55,56,58]. This is at least partly due to the increased prevalence of poor risk features in adults. In the RAH cohort, only one of twelve patients with ALL had good prognostic features at presentation. Forty-two percent were positive for the Philadelphia translocation. A high proportion adult ALL (29%) positive for the Philadelphia chromosome was also reported by the Groupe Francais de Cytogenetique Hematologique [11], who also found a very low percentage of adults with ALL presenting with good risk features.

The role of transplantation in adult ALL has been well analysed. The International Bone Marrow Transplant Registry reported 9-year disease free survival rates of 34% for sibling allogeneic transplantation compared to 32% if given chemotherapy in first CR [59,60]. The LALA87 trial reported similar 3 year survival rates of 43%, 39%, 32% for allogeneic transplantation, autologous transplantation, and chemotherapy respectively [61-63]. These survival rates were not statistically different. Although there was no OS advantage for the 98 patients who had an allogeneic transplantation in first CR, a survival advantage was found for high-risk patients, with a 5-year survival of 39% compared to 14% [59] .

The results of the LALA94 trial are now known. Overall, of a total of 922 patients, 771 patients achieved CR (84%). Median DFS was 17.5 months, with 3-year DFS at 37%. In the standard-risk group 1 the median OS was 37.8 months, with 3-year OS at 50% and 5-year OS at 44%. There were 307 patients who were randomly assigned and received post-induction therapy: one hundred and fifty three were randomly assigned to early intensive chemotherapy and 154 were randomly assigned to chemotherapy without early intensification. The 3-year DFS rate was 41%, with no difference between arms of post-remission randomisation. In the high-risk (excluding Philadelphia positive) and CNS-positive group, the 3-year DFS rates were 38% and 44%, respectively. Those patients with an HLA-matched sibling had improved DFS, with median OS 29 months and 3-year OS at 46% and 5-year OS at 38%. Eighty-two patients with a sibling donor were scheduled for allogeneic SCT. In intention-to-treat analysis, autologous SCT and chemotherapy produced comparable median DFS (15.2 versus 11 months, respectively). However, late relapses were more frequent in the chemotherapy arm (3-year DFS rate, 39% v 24%). Median OS was 28 months with 3-year OS at 44% and 5-year OS at 32% in the autologous SCT arm versus 26.1 months with 3-year OS at 35% and 5-year OS at 21% in the chemotherapy arm.

For high-risk ALL on intention-to-treat, the outcome for patients who received allogeneic transplantation was better than the other groups in terms of DFS. The median DFS was 20.8 months

with a 5-year DFS rate of 44%, whereas the median OS was not reached with a 5-year OS rate of 51%. For Philadelphia-positive and/or BCR-ABL–positive ALL, the outcome was poor, with a median OS of 15.7 months with 3-year OS rate of 28% and a 5-year OS rate of 24%. Median OS was 14.2 months for autografted patients and 21.5 months for allografted patients (including 10 matched unrelated donor SCT) with 3-year OS rates at 17% and 36%, respectively. The trial investigators concluded that allogeneic SCT improved DFS in high-risk ALL in the first CR. Autologous SCT did not confer a significant benefit over chemotherapy for high-risk ALL.

Using standard ALL treatment, B ALL L3 morphology was previously regarded as having a poor prognosis [64]. However, during the 1990s the outlook has improved considerably following the introduction of intensive treatment regimens, and this subtype of leukemia may now be regarded as good risk for younger patients [58,65-69]. However, for older patients the prognosis has not improved [70]. New forms of therapy and remain the best hope for such patients.

6. NOVEL MOLECULAR TARGETED BASED APPROACHES TO THERAPY

Advances in the understanding of the molecular basis of acute leukemia have led to the development of new therapies targeting key oncogenic pathways. Examples of recent major development include inhibitors of ABL, FLT3, VEGF, DNA methyltransferase and histone deacetylase, as well as conjugated anti-CD33 monoclonal antibodies.

6.1. BCR-ABL TYROSINE KINASE INHIBITOR

Selective BCR-ABL tyrosine kinase inhibitor imatinib has shown clinical activity in chronic myeloid leukemia (CP) as well as blast phase (BP), and has also shown some activity in Philadelphia-positive ALL [71-74]. In two recently reported large phase II studies, 55% of patients with myeloid BP achieved complete hematologic response lasting a median of 10 months. The median survival and 1-year survival rates were approximately 7 months and 30%, respectively [75,76]. The role of imatinib in lymphoid BP is less established than in myeloid BP. The response rate is 30–70% but the median remission duration is only 2 months [72]. Improved understanding of the molecular mechanisms of resistance have aided in the development of novel agents which overcome imatinib resistance [77]. Crystallographic studies of imatinib-resistant mutants have led to the development of drugs that overcome acquired resistance to imatinib. BMS-354825 and AMN107 are orally bioavailable ABL kinase inhibitors with significantly increased potency relative to imatinib, and retain activity against imatinib-resistant BCR-ABL mutants [78,79]. These novel therapies demonstrate the accelerated pace of drug development based upon improved understanding of not only the molecular pathogenesis of disease, but also of subsequent drug resistance.

6.2. INTERNAL TANDEM DUPLICATION INHIBITORS

An internal tandem duplication (ITD) in the FLT3 gene resulting in constitutively activating FLT3 receptor has recently been described [80-86]. It is so far the commonest mutation described in AML. Seventy percent of patients have an otherwise normal karyotype. It has been correlated with a high peripheral blood count in FAB M1, M2, and M4. The United Kingdom Medical Research Council (MRC) AML trials reported that although the effect of an FLT3 mutation did not differ between the cytogenetic risk groups, FLT3 mutation was associated with a lower CR rate and a higher induction death rate, as well as with increased relapse risk, adverse DFS, event-free survival, and OS ($P < 0.001$ for all) [87]. Within the AMLCG study, the FLT3 mutation was of intermediate prognostic significance. The CR rate of 70% in patients with FLT3 mutation was similar (70%) to that in patients without FLT3

mutation. Overall survival was not different between patients with or without FLT3 mutation [88]. The FLT3/ITD mutation discovery has translated directly into potential future therapy. A small molecule FLT3 tyrosine kinase inhibitor PKC412 has been shown to selectively induce G1 arrest and apoptosis of Ba/F3 cell lines expressing mutant FLT3 (IC_{50} < 10 nM) by directly inhibiting the tyrosine kinase. PKC412 also prevented progressive leukemia in PKC412-treated Balb/c mice transplanted with marrow transduced with an FLT3/ITD-expressing retrovirus [89].

6.3. DNA METHYLTRANSFERASE INHIBITORS

In cancer cells, the DNA-methylation and chromatin patterns are different from normal cells [90,91], and a growing list of genes have been identified as having abnormal methylation of promoters, with associated transcriptional silencing [92]. Transcriptional repression by hypermethylation of promoter sequences may inactivate tumour-suppressor genes, such as the retinoblastoma gene. A family of enzymes, the DNA methyltransferases (DNMTs), catalyze the DNA methylation, and this reaction can be blocked by the cytidine analogue drugs 5-azacytidine (Aza C) and 2-deoxy 5-azacytidine (decitabine). 5-azacytidine was approved by the US FDA in May 2004 for use in myelodysplastic syndrome. A CALGB trial of Aza C in myelodysplasia reported a median survival of 20 months for patients randomized to Aza C compared with 14 months for patients undergoing supportive care (53% of whom received Aza C after cross-over) [93]. There is molecular evidence for using demethylating agents for the treatment of acute leukemia [94-98]. In a phase 1 study of low-dose prolonged schedules of decitabine in relapsed/refractory leukemias, a total of 50 patients were treated, including 44 with AML/myelodysplasia, 5 with CML, and 1 with ALL. The drug was well tolerated at all dose levels, and responses were seen at all dose levels. Interestingly, most responses were seen at less than maximal tolerated doses (11 of 17 or 65%), with fewer responses seen when the dose was escalated or prolonged (2 of 19 or 11%) [99] possibly reflecting the predominant mechanism of action of these drugs is via up-regulation of tumour suppressor genes and anti-oncogenes, rather than inhibition of DNA replication.

6.4. HISTONE DEACETYLASE INHIBITORS

Histone deacetylase (HDAC) inhibitors are a novel class of compounds able to regulate gene expression by modulating chromatin structure. HDAC inhibitors affect differentiation and proliferation, and they also induce apoptosis in tumour cells. A new hydroxamic acid derivative, NVP-LAQ824 [100-103], significantly inhibited the proliferation of leukemic lymphoblastic cell lines [104], as well as myeloid blast cells [105,106] and is effective in a murine model of AML [107]. In a phase 1 clinical trial, 21 patients (median age: 68 yrs) with AML (15 patients), MDS (4 patients) and CLL (2 patients) have been treated with escalating doses of NVP-LAQ824. One patient with de novo M1 AML achieved a CR after 2 cycles, and remained in CR after 6 cycles. Six patients had stable disease or hematologic improvement [108]. NVP-LAQ824 is also synergistic with VEGF inhibitor PTK787 [109] as well as Apo-2L/tumor necrosis factor-related apoptosis inducing ligand-induced death inducing signaling complex activity [110].

6.5. VASCULAR ENDOTHELIAL GROWTH FACTOR INHIBITORS

Vascular endothelial growth factor (VEGF) is expressed in leukemic blasts, and is a potential new target for therapy of acute leukemia. VEGF promotes AML cell growth and survival and may contribute to drug resistance. Bevacizumab is an inhibitor of VEGF tyrosine kinase inhibitor which has achieved responses in adults with AML that is resistant to traditional treatment approaches [111]. In a Phase II clinical trial of bevacizumab administered after chemotherapy to adults with

refractory or relapsed AML, the overall response was 23 of 48 (48%), with CR in 16 (33%). Median overall and disease-free survivals for CR patients were 16.2 months (64% at 1 year) and 7 months (35% at 1 year). Marrow blasts demonstrated FLT-1 staining before bevacizumab and marked decrease in microvessel density after bevacizumab. VEGF was detected in pre-treatment serum in 67% of patients tested, increased by day 8 in 52%, and decreased in 93% (67% undetectable) 2 hours after bevacizumab treatment. These results indicated a favorable CR rate and duration in adults with AML that is resistant to traditional treatment approaches.

6.6. ANTI-CD33 MONOCLONAL ANTIBODIES

Myeloid blast cells express surface CD33, whereas normal hematopoietic stem cells do not. Therefore, anti-CD33 monoclonal antibodies have been developed for use in CD33 positive AML. Gemtuzumab is an anti-CD33 monoclonal antibody conjugated to a calicheamicin-gamma derivative, and was approved for clinical use in 2000 [112]. Gemtuzumab binds to CD33, resulting in immunoconjugate internalization and hydrolytic release of the toxic calicheamicin moiety, which, in turn, causes DNA damage and cell death [113]. Gemtuzumab has shown efficacy as a single agent in AML and myeloid sarcomas [114-117]. Furthermore, Gemtuzumab has single agent activity in APML [118], and has been successfully used to enhance the activity of other therapies including ATRA [119-121].

7. CONCLUSION

Acute leukemia is a heterogeneous disease. As molecular and cytogenetic techniques improve, the ability to subtype leukemia for prognostic and therapeutic purposes becomes greater. This has implications for new chemotherapy, and a better ability to advise patients about the risks and benefits of extremely toxic therapy such as allogeneic transplantation. The development of new forms of therapy based upon molecular understanding of the malignant clone gives hope for future sufferers of acute leukemia.

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