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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig S1. $CD4^+$ T cells are highly effective inducers of CLL cell proliferation.

Fig S2. Analysis of proliferative potential of CLL cells in *in vitro* cultures with respect to common risk factors.

References

Bagnara, D., Kaufman, M.S., Calissano, C., Marsilio, S., Patten, P.E., Simone, R., Chum, P., Yan, X.J., Allen, S.L., Kolitz, J.E., Baskar, S., Rader, C., Mellstedt, H., Rabbani, H., Lee, A., Gregersen, P.K., Rai, K.R. & Chiorazzi, N. (2011) A novel adoptive transfer model of chronic lymphocytic leukemia suggests a key role for T lymphocytes in the disease. *Blood*, **117**, 5463–5472.

Brachtl, G., Sahakyan, K., Denk, U., Girbl, T., Alinger, B., Hofbauer, S.W., Neureiter, D., Hofbauer, J.P., Egle, A., Greil, R. & Hartmann, T.N. (2011) Differential bone marrow homing capacity of VLA-4 and CD38 high expressing chronic lymphocytic leukemia cells. *PLoS ONE*, **6**, e23758.

Burger, J.A., Ghia, P., Rosenwald, A. & Caligaris-Cappio, F. (2009) The microenvironment in mature B-cell malignancies: a target for new treatment strategies. *Blood*, **114**, 3367–3375.

Hallaert, D.Y., Jaspers, A., van Noesel, C.J., van Oers, M.H., Kater, A.P. & Eldering, E. (2008) c-Abl kinase inhibitors overcome CD40-mediated drug resistance in CLL: implications for therapeutic targeting of chemoresistant niches. *Blood*, **112**, 5141–5149.

Hamilton, E., Pearce, L., Morgan, L., Robinson, S., Ware, V., Brennan, P., Thomas, N.S., Yallop, D., Devereux, S., Fegan, C., Buggins, A.G. & Pepper, C. (2012) Mimicking the tumour microenvironment: three different co-culture systems induce a similar phenotype but distinct proliferative signals in primary chronic lymphocytic leukaemia cells. *British Journal of Haematology*, **158**, 589–599.

Herishanu, Y., Perez-Galan, P., Liu, D., Biancotto, A., Pittaluga, S., Vire, B., Gibellini, F., Njuguna, N., Lee, E., Stennett, L., Raghavachari, N., Liu, P., McCoy, J.P., Raffeld, M., Stetler-Stevenson, M., Yuan, C., Sherry, R., Arthur, D.C., Maric, I., White, T., Marti, G.E., Munson, P., Wilson, W. H. & Wiestner, A. (2011) The lymph node microenvironment promotes B-cell receptor signaling, NF-kappaB activation, and tumor proliferation in chronic lymphocytic leukemia. *Blood*, **117**, 563–574.

Hofbauer, J.P., Heyder, C., Denk, U., Kocher, T., Holler, C., Trapin, D., Asslaber, D., Tinhofer, I., Greil, R. & Egle, A. (2011) Development of CLL in the TCL1 transgenic mouse model is associated with severe skewing of the T-cell compartment homologous to human CLL. *Leukemia*, **25**, 1452–1458.

Messmer, B.T., Messmer, D., Allen, S.L., Kolitz, J. E., Kudalkar, P., Cesar, D., Murphy, E.J., Koduru, P., Ferrarini, M., Zupo, S., Cutrona, G., Damle, R.N., Wasil, T., Rai, K.R., Hellerstein, M.K. & Chiorazzi, N. (2005) *In vivo* measurements document the dynamic cellular kinetics of chronic lymphocytic leukemia B cells. *J Clin Invest*, **115**, 755–764.

Patten, P.E., Buggins, A.G., Richards, J., Wotherspoon, A., Salisbury, J., Mufti, G.J., Hamblin, T. J. & Devereux, S. (2008) CD38 expression in chronic lymphocytic leukemia is regulated by the tumor microenvironment. *Blood*, **111**, 5173–5181.

Plander, M., Seegers, S., Ugocsai, P., Diermeier-Daucher, S., Ivanyi, J., Schmitz, G., Hofstadter, F., Schwarz, S., Orso, E., Knuchel, R. & Brockhoff, G. (2009) Different proliferative and survival capacity of CLL-cells in a newly established *in vitro* model for pseudofollicles. *Leukemia*, **23**, 2118–2128.

A phase 2 study of SRT501 (resveratrol) with bortezomib for patients with relapsed and or refractory multiple myeloma

Resveratrol (a naturally occurring phytoalexin produced by plants) has described cancer prevention properties and is potentially responsible for the disease-preventing properties of red wine (Jang *et al*, 1997; Baur & Sinclair, 2006). It has poor water solubility and low bioavailability, hence SRT501 (a micronized oral formulation) was developed and subsequently demonstrated to activate Sirtuin Enzyme 1 (SIRT1) (Sirtris Pharmaceuticals, Inc, 2009). Over-expression of SIRT1 inhibited cancer cell growth (Michishita *et al*, 2005), reduced cell invasion, and regulated cell cycle and DNA replication. Resveratrol demonstrated cytotoxicity in multiple myeloma (MM) cell lines and inhibited nuclear factor kappa B, as well as AKT and Signal transducer and activator of transcription 3 (Jazirehi & Bonavida, 2004). The combination of resveratrol and bortezomib achieved synergistic cytotoxicity *in vitro* (Bhardwaj *et al*, 2007) providing rational for clinical development.

Twenty four patients (median age 66·5 years) were enrolled into this phase 2 clinical trial of SRT501 with or without bortezomib in MM patients who had relapsed or were refractory to at least one prior therapy. Inclusion criteria included adequate renal/bone marrow function, and

Table I. Response rates as per European Group for Blood and Marrow Transplant criteria (Blade *et al*, 1998) for patients enrolled on study. Both the protocol-defined modified intention-to-treat (completing the first two cycles of SRT501monotherapy) and an intention-to-treat analysis (all patients) are shown. Responses are separated according to treatment received.

	Protocol-defined modified Intent-to-treat analysis*		Intent-to-treat analysis
Response, *n* (%)	SRT501 monotherapy† *n* = 13	SRT501 + bortezomib‡ *n* = 9	SRT501 + bortezomib‡ *n* = 24
Overall response rate	0	2 (22)	2 (8)
Complete response	0	0	0
Partial response	0	1 (11)	1 (4)
Minor response	0	1 (11)	1 (4)
Stable disease	9 (69)§	3 (33)	3 (13)
Progressive disease	4 (31)	4 (44)	4 (8)

*Received $\geq$2 cycles of SRT501 monotherapy.

†Best response achieved during monotherapy portion; 9 subjects crossed over to SRT501 + Bortezomib.

‡Best response from $\geq$Cycle 3 or beyond.

§4/9 SD only received 2 cycles.

Table II. Biochemistry and further investigation parameters for the five patients that developed renal failure. Not all patients had a renal biopsy, in these cases ultrasound findings are presented and efficacy data.

Patient	Age (years)	Serum creatinine at trial entry (μmol/l)	Serum creatinine at SAE (μmol/l)	Further investigations
1	72	102	697	Renal biopsy: Acute tubular injury, no casts seen
2	74	181	539	Renal biopsy: Cast nephropathy
3	48	114	618	No renal biopsy performed Paraprotein assessments: Trial entry: 78·69 g/l At time of SAE: 92 g/l Renal ultrasound scan: Echogenic kidneys with loss of differentiation
4	67	122	765	No renal biopsy performedRenal ultrasound scan: Echogenic kidneys with loss of differentiation
5	76	73	900	No renal biopsy performed K light chain assessments: Trial entry: 370 mg/l At time of SAE: 1482 mg/l

SAE, serious adverse event.

prior bortezomib was permitted irrespective of response. Patients received 5·0 g of SRT501 (Sirtris Pharmaceuticals Inc., Cambridge, MA, USA) following breakfast for 20 d in a 21-d cycle up to 12 cycles. After two cycles, those with $\geq$stable disease (SD) received two additional cycles, those with progressive disease (PD) had bortezomib added (1·3 mg/m^2 on days 1,4,8, and 11). Patients achieving $\geq$SD after four cycles monotherapy continued SRT501 until PD whereupon bortezomib was added. Those with PD during SRT501 and bortezomib were withdrawn. 40 subjects were planned to result in 30 evaluable patients (defined as completing the first two cycles of SRT501monotherapy) with an estimated overall response rate (ORR) of 35% according to European Group for Blood and Marrow Transplant criteria (Blade *et al*, 1998). Patients had received a median of 4 (range 1–9) prior therapies, 19 had prior bortezomib of which 14 had responded. Prior treatment combinations were: thalidomide (71%), bortezomib (88%), lenalidomide (58%), autograft (75%), cyclosphosphamide (38%), melphalan (17%), others (21%). All 24 patients commenced SRT501 monotherapy, and nine had bortezomib added for PD. Two protocol violations occurred, resulting in exclusion from efficacy (but not safety) analysis. The mean duration on study was 92·8 d (range 1–351) and patients received a mean cumulative dose of 336 g SRT501 (range 5–1505 g).

Eleven patients discontinued before first response assessment and, according to study protocol, were not evaluable. The modified intention-to-treat population (mITT) comprised 13 patients. The median number of cycles was two (monotherapy) and three additional cycles for SRT501 and bortezomib. 10 (42%) subjects continued with SRT501 monotherapy beyond cycle 2, and 4 received only SRT501 monotherapy (maximum eight cycles); however none receiving monotherapy achieved $\geq$SD. Nine (69%) of 13 evaluable subjects had SD following a minimum of two cycles, but as four crossed over to the SRT501 + bortezomib arm, durability could not be assessed. Nine patients received SRT501 and bortezomib combination, achieving an ORR ($\geq$MR) by mITT of 22% or, by intention-to-treat analysis, an ORR of 8% (Table I). Six of nine patients treated with the combination continued for more than two cycles, up to a maximum of eight cycles. The median time to progression was 2·8 months and overall survival was not reached.

The most commonly reported adverse events [AEs, National Cancer Institute Common Toxicity Criteria v4·0 (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4·03_2010-06-14_QuickReference_8·5x11.pdf)] were: nausea (79%), diarrhoea (71%), vomiting (54%), fatigue (46%) and anaemia (38%). 54% of patients reported $\geq$ grade 3 AEs; most commonly 21% haematological (anaemia and thrombocytopenia), 21% renal failure, 13% nausea and 13% infections. 50% had a serious AE (SAE) and two deaths occurred on study (one possibly treatment related; one due to PD). 15 of 24 patients treated with SRT501 monotherapy were withdrawn from treatment (10 due to AEs, 1 due to AE leading to death and 4 following investigator's decision). All nine patients receiving SRT501 and bortezomib were withdrawn from study (5 due to AEs, 3 following investigator's decision and one patient withdrawal). The predominant study finding was unexpected renal toxicity, with five SAEs of renal failure leading to early study termination (Table II). Three of these five patients had an elevated serum creatinine prior to first dose and 4 of the 5 were taking long-term sodium clodronate. Renal failure occurred within the first two cycles of SRT501 monotherapy, with a median time from first dose to renal impairment of 7 d (range 1–37); however one patient had an increase in creatinine before treatment. All five patients reported nausea and vomiting prior to hospitalization and two required temporary haemodialysis. Renal biopsy performed in two patients demonstrated cast and crystal nephropathy in one, and acute tubular damage without cast nephropathy in the other. Disease progression occurred in four of the five patients at the time of renal failure and two patients were discharged for palliation. Following three cases, a medical review meeting recommended further monitoring and recruitment was stopped following five SAEs.

Two hundred and thirty seven patients in seven studies had previous SRT501 treatment, predominantly healthy volunteers (n = 92), but also type two diabetes (n = 136) and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome. The phase 2 dose (5·0 g) was safely assessed in these studies (Sirtris Pharmaceuticals, Inc, 2009). Furthermore, a Phase 1 study for metastatic colorectal cancer patients (Howells *et al*, 2011), did not report nephrotoxicity. As SRT501 is extensively metabolized, renal failure seemed specific to MM patients. Renal impairment can occur in up to 50% of MM patients (Bird *et al*, 2011) due to multiple causes, hence all are at risk. Three of these patients had an elevated serum creatinine at screening, and rose in another prior to treatment, suggesting pre-existing renal damage. Furthermore, four patients demonstrated progressive myeloma at SAE. Renal failure was not observed for SRT501 and bortezomib despite low efficacy (14 had prior bortezomib and five not previously responded). However disease stabilization by bortezomib may have prevented renal failure whereas low efficacy of SRT501 with nausea and vomiting may have resulted in disease progression and dehydration, leading to renal failure. This study demonstrated an unacceptable safety profile and minimal efficacy in patients with relapsed/refractory MM highlighting the risks of novel drug development in such populations.

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This study was registered at www.ClinicalTrials.gov: NCT00920556 and was approved by local ethics boards, compliant with declaration of Helsinki and informed consent obtained for all patients.

All authors were involved in conducting the study, collecting trial data, writing and approving the final manuscript. JC and EJ designed the study, RP, EJ and JC analysed the data. PE and EJ are/ were employees of Sirtris, A GSK Company at time of study. TP, FD and RP have received honorarium from Janssen.

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References

Baur, J.A. & Sinclair, D.A. (2006) Therapeutic potential of resveratrol: the *in vivo* evidence. *Nature Reviews Drug Discovery*, **5**, 493–506.

Bhardwaj, A., Sethi, G., Vadhan-Raj, S., Bueso-Ramos, C., Takada, Y., Gaur, U., Nair, A.S., Shishodia, S. & Aggarwal, B.B. (2007) Resveratrol inhibits proliferation, induces apoptosis, and overcomes chemoresistance through down-regulation of STAT3 and nuclear factor-κB regulated antiapoptotic and cell survival gene products in human multiple myeloma cells. *Blood*, **109**, 2293–2302.

Bird, J.M., Owen, R.G., D'Sa, S., Snowden, J.A., Pratt, G., Ashcroft, J., Yong, K., Cook, G., Feyler, S., Davies, F., Morgan, G., Cavenagh, J., Low, E. & Behrens, J. (2011) Guidelines for the diagnosis and management of multiple myeloma 2011. *British Journal of Haematology*, **154**, 32–75.

Blade, J., Samson, D., Reece, D., Apperley, J., Bjorkstrand, B., Gahrton, G., Gertz, M., Giralt, S., Jagannath, S. & Vesole, D. (1998) Criteria for

evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation. Myeloma Subcommittee of the EBMT. European Group for Blood and Marrow Transplant. *British Journal of Haematology*, **102**, 1115–1123.

Howells, L.M., Berry, D.P., Elliott, P.J., Jacobson, E.W., Hoffmann, E., Hegarty, B., Brown, K., Steward, W.P. & Gescher, A.J. (2011) Phase I randomized, double-blind pilot study of micronized resveratrol (SRT501) in patients with hepatic metastases–safety, pharmacokinetics, and pharmacodynamics. *Cancer Prevention Research (Philadelphia, Pa.)*, **4**, 1419–1425.

Jang, M., Cai, L., Udeani, G.O., Slowing, K.V., Thomas, C.F., Beecher, C.W., Fong, H.H., Farnsworth, N.R., Kinghorn, A.D., Mehta, R.G., Moon, R.C. & Pezzuto, J.M. (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*, **275**, 218–220.

Jazirehi, A.R. & Bonavida, B. (2004) Resveratrol modifies the expression of apoptotic regulatory proteins and sensitizes non-Hodgkin's lymphoma and multiple myeloma cell lines to paclitaxel-induced apoptosis. *Molecular Cancer Therapeutics*, **3**, 71–84.

Michishita, E., Park, J.Y., Burneskis, J.M., Barrett, J.C. & Horikawa, I. (2005) Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. *Molecular Biology of the Cell*, **16**, 4623–4635.

Sirtris Pharmaceuticals, Inc. (2009) *SRT501 Investigator's Brochure Oncology*. Sirtris Pharmaceuticals, Inc., Cambridge, MA, USA.

A procoagulant state and endothelial dysfunction in obese children

Singh and colleagues recently found tissue factor procoagulant activity to be 78% higher in 21 obese children than in 22 healthy-weight sex and age-matched children (Singh *et al*, 2012). They also found Factor VII coagulant activity to be 14% higher, and that levels of plasminogen activator inhibitor-1 (PAI-1) were some 47% higher. They use this data to conclude that obese children are in a procoagulant state. Whilst this conclusion is not unreasonable, they have measured 12 laboratory indices in a sample size of only 43 subjects, leading to fears of false positives and false negatives, pertinent in a study with no clear original hypothesis or power calculations. I have three observations.

Close attention to the data in Table I reveals that levels of PAI-1 in the healthy weight group to be mean 25·6 ng/ml with a standard deviation of 15 ng/ml. Given that the mean $\pm$ two standard deviations includes 95% of data points, arithmetic demands that some children must have a negative PAI-1 result – clearly impossible. As this implies that data has a non-normal distribution, perhaps presentation as median with interquartile range would obviate this point and, accordingly, analysis by the Mann–Whitney *U* test would give a different p value. The same argument applies to the soluble vascular adhesion molecule 1 (sVCAM1) data, which appears to have a non-normal distribution (simple rule of thumb standard deviation >50% of the mean) in the obese children.

Singh *et al* (2012) also claim, in their abstract and discussion, that there is endothelial dysfunction, presumably based on the von Willebrand factor (VWF), PAI-1 and sVCAM data. Inclusion of the VWF data in this setting is curious because, according to Table I of the report, there is no statistically significant difference between the groups. In addition, it is well known that that ABO blood group can account for some 20% of the variance of this molecule (Stormorken & Erikssen, 1977) and therefore must be adjusted for in small studies such as this. It is entirely feasible that differences in ABO blood group could account for failure to find a difference in plasma VWF if all the children had the same ABO status. The use of PAI-1 as a vascular marker must be tempered by the established fact that levels of this molecule can arise from many cells, including the platelet (Torr-Brown & Sobel, 1993), monocytes (Castellote *et al*, 1990) and adipocytes (Morange *et al*, 1999; Eriksson *et al*, 2000). Similarly, VCAM1 is widely expressed not only on endothelial cells, but also on epitheial and dendritic cells (Gearing & Newman, 1993), macrophages and smooth muscle cells (Braun *et al*, 1995). Indeed, in the abstract to a review cited by Singh *et al* (2012), Mertens and Van Gaal (2002) state that 'it has been demonstrated that the adipocyte itself is able to produce PAI-1, possibly explaining the high levels found in obesity'. It follows that use of PAI-1 and VCAM1 as plasma markers of endothelial dysfunction/perturbation is unsound.

According to Machin and Campbell (1987), a sample size of 46 brings the 1-beta of 0·8 to robustly defend a correlation coefficient of 0·4 at $P < 0{\cdot}05$. Whilst the sample size of Singh *et al* (2012) exceed this by one, the correlation coefficients in their Fig 1, 0·36–0·38, are all less than 0·4. A sample size of at least 61 is required to minimize risks of false positive and negatives (that is, $2P < 0{\cdot}05$ and 1-beta = 0·8) in the presence of a correlation coefficient >0·35 but <0·4.

I therefore refute the assertion of Singh *et al* (2012) that their data supports the hypothesis that there is evidence of procoagulant state and endothelial dysfunction in obese children.

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