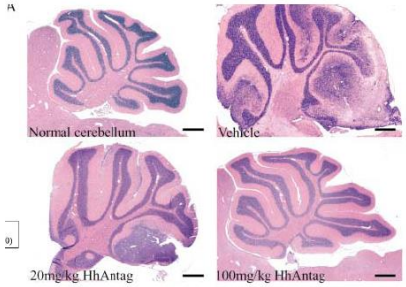
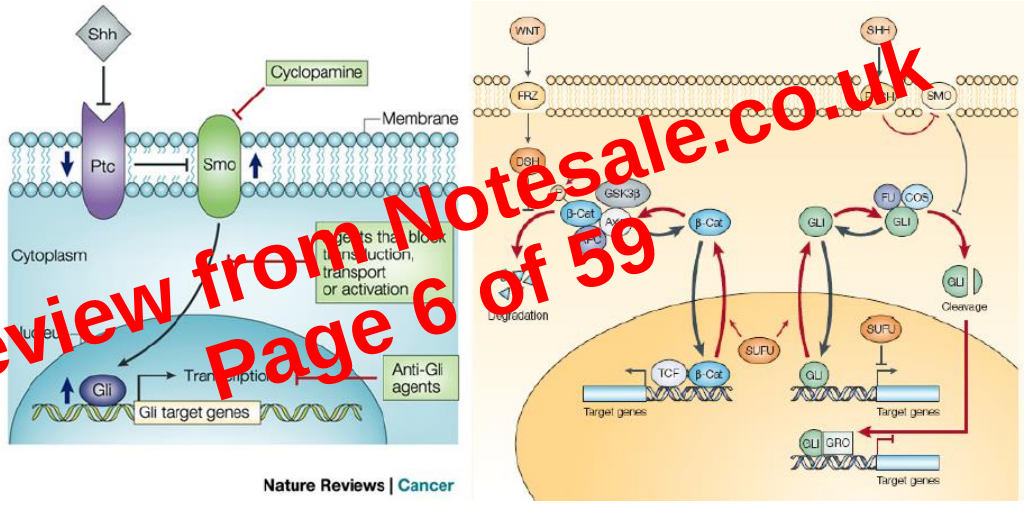
- Develop new drug target:
    - Understand relationship between tumour cells and normal cells.
        - What's gone wrong – global analyses.
    - Develop agents that kills cells – either new agents or overcome problems of old agents.
    - Medulloblastoma – many tumours have activated shh pathway, so block shh receptor.
- Suppression of the Shh pathway using a small molecule inhibitor eliminates medulloblastoma in Ptc+/- p53-/- mice:
    - ShhAntag-691 a Smo inhibitor led to:
        - Elimination of tumours.
        - Decrease in tumour cell proliferation.
        - Increase in apoptosis.
        - Treatment prolonged tumour free survival.
    - Most trials now for adult non-brain tumours.



- So far smo inhibitors are used regardless of profiling to see where mutations are – SUFU vs. PTCH/Smo:
    - Infants good target but also probably cause developmental defects.

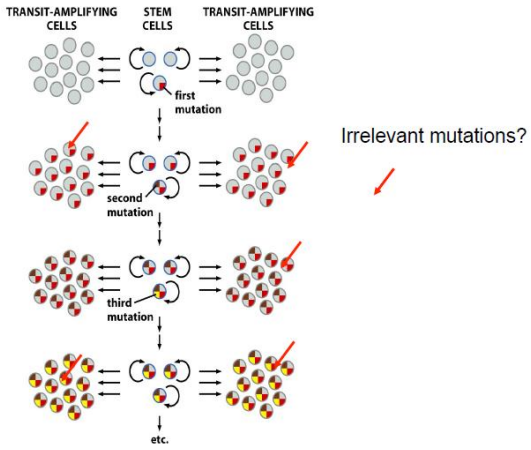


- Glial tumours:
    - Grade I – benign.
    - Grade II – slow but not caused by surgery.
    - Grade III – death in a few years.
    - Grade IV – GBM (Glioblastoma multiforme), death within months (<5% make 5 yrs).
    - II – convert to III and IV after several years.

populations, notably promoter methylation events, can play an equally prominent role in multi-step tumor progression.

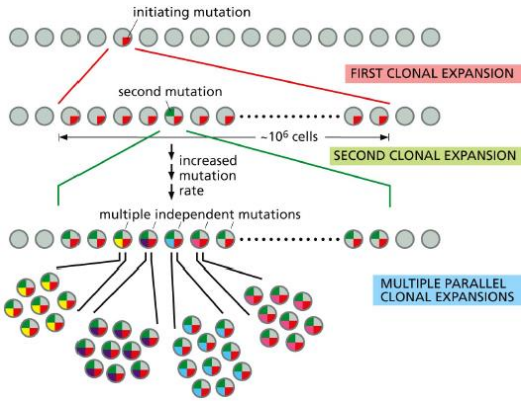
- Importantly, this scheme does not take into account the fact that clonal successions may require greatly different time intervals to reach completion.
  - For example, later successions are likely to proceed far more rapidly than earlier ones because the participating cells, having acquired oncogenic mutations, may proliferate more rapidly and have more mutable genomes.

- Cancer stem cells and the evolution of cancers:

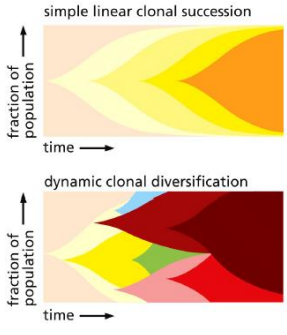


  - The existence of cancer stem cells has important implications for how tumour progression and clonal succession occur.
  - Here, less differentiated cells (i.e. cancer stem cells) can differentiate into transit-amplifying cells, but the reverse process (dedifferentiation of transit-amplifying cells into cancer stem cells) does not occur.
  - Moreover, the cancer stem cells represent a minority of the neoplastic cells in tumour masses, while the transit-amplifying cells represent the majority of neoplastic cells.
  - Therefore, mutations that strike the genomes of tumour stem cells can be transmitted to descendent tumour stem cells, which can then launch new clonal successions.
  - Conversely, mutations that strike the genomes of transit-amplifying cells cannot be transmitted further because these cells have only limited replicative ability.
    - An exception to this scheme may occur if a mutation striking a transit-amplifying cell converts it into a stem cell.

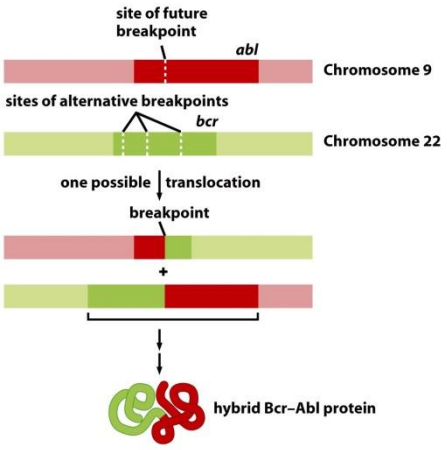
- Cancer stem cells and the evolution of cancers?



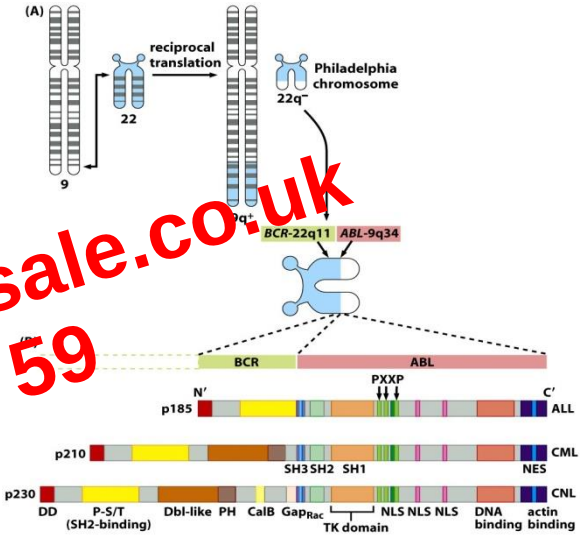
  - As tumour progression proceeds, the genomes of tumour cells often become increasingly unstable.
  - As this occurs, the rate at which new mutant alleles are generated may exceed the rate at which Darwinian selection can eliminate phenotypically lessfit clones.
  - Consequently, the tumour mass develops an increasing number of distinct sectors, each dominated by a genetically distinct sub-clone.
  - In this diagram, the involvement of transit-amplifying and cancer stem cells in these processes is not indicated.
  - Tumours are made up of genetically distinct subclones, several of which might be able to function as CSCs/TICs.
  - Recent surveys of both genetic and epigenetic (CpG methylation) heterogeneity within individual tumours have provided experimental support for the extensive diversification of distinct clonal populations within a tumour.



- Reciprocal chromosomal translocations between human Chromosomes 9 and 22, which carry the abl and bcr genes, respectively, result in the formation of fused, hybrid genes that encode hybrid Bcr–Abl proteins commonly found in chronic myelogenous leukemias (CML).
    - Two other alternative breakpoint sites in the bcr gene are involved in bcr–abl translocations (not shown here) arising in certain other hematopoietic malignancies.

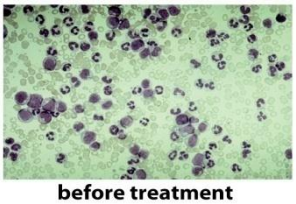


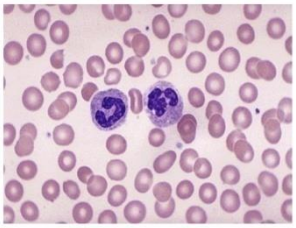
- Philadelphia translocations:
    - *bcr/abl* fusion can activate:
        - The Ras pathway, PI3-kinase pathway, Jak-STAT pathway.
        - Transcription factors including Jun, Myc and NF-$\varkappa\beta$.
            - Cell migration, survival and proliferation.
        - Most important phenotype is tyrosine kinase signaling.
- Depending on the bcr region where the break occurs, three fusion proteins are produced:
    - Can happen in different cell types but only oncogenic in particular cell types.
    - Translocations might be entirely random – only reason you see them in leukaemia is because they generate a product which gives that cell a selective advantage.
    - AML, CML, and CNL proteins produced.
    - More than 95% of cases of chronic myelogenous leukemia (CML) exhibit the Philadelphia chromosome, which results from a reciprocal translocation between Chromosomes 9 and 22.



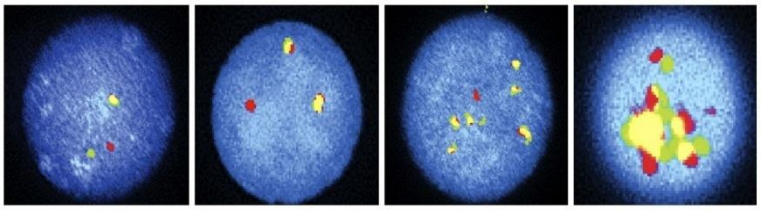
        - The q34 region of Chromosome 9 carrying most of the ABL gene is transferred to the q11 region of Chromosome 22, replacing a larger segment of Chromosome 22 that is translocated reciprocally to Chromosome 9.
        - The net result is a truncated Chromosome 22 (i.e., 22q–), often termed the Philadelphia chromosome (Ph), and a fusion of the 5′ portion of the ABL gene with a 3′-proximal portion of the BCR gene, which normally resides at 22q11.
    - Depending on the precise location of the breakpoint in BCR, three distinct Bcr-Abl fusion proteins may be formed; these are found in ALL (acute lymphoblastic leukemia), CML, and CNL (chronic neutrophilic leukemia).
        - Each of these BCR-ABL fusion genes encodes a multidomain (and thus multifunctional) protein.

- The patient:
    - Treatment:
        - Chemotherapy:
            - Actively dividing cells.
            - Massive doses required.
        - Radiotherapy:
            - Used if leukaemia has spread to the CNS.
            - Used in Bone Marrow/Stem Cell Transplant.
    - Must replace the stem cells to restore heamatopoietic system to patient.
        - Bone Marrow or Stem Cell Transplant


before treatment

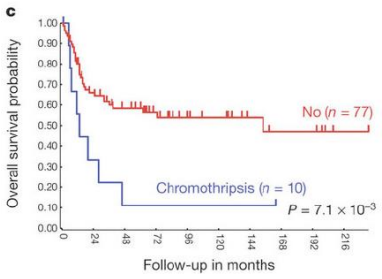

after treatment

- Biologically targeted therapy – treating the Philadelphia translocation:
    - A low molecular weight antagonist of the Bcr-Abl tyrosine kinase has been developed.
        - Gleevec/imatinib mesylate/glivec.
        - Inhibits only 4 of the 90 human tyrosine kinases.
    - Clinical trials in 1998 demonstrated remission in all 31 treated CML patients.
    - By 2002 6000 patients were in Gleevec clinical trials.
- Treatment problems:
    - 90% of patients respond positively.
    - 50% patients, translocation is lost.
    - But, 60% of patients who have already progressed to blast crisis respond, but subsequently relapse.
        - Blast crisis = final phase when >30% cells are leukaemic and progression is very rapid.
    - Relapsing patients:
        - 29/32 patients have new mutations in the BCR-ABL gene prevent Gleevec binding to catalytic cleft.
    - Minority – upregulation of BCR-ABL gene.
    - Treatment reduces BCR-abl transcripts.
        - But then gene is amplified so number goes up again.

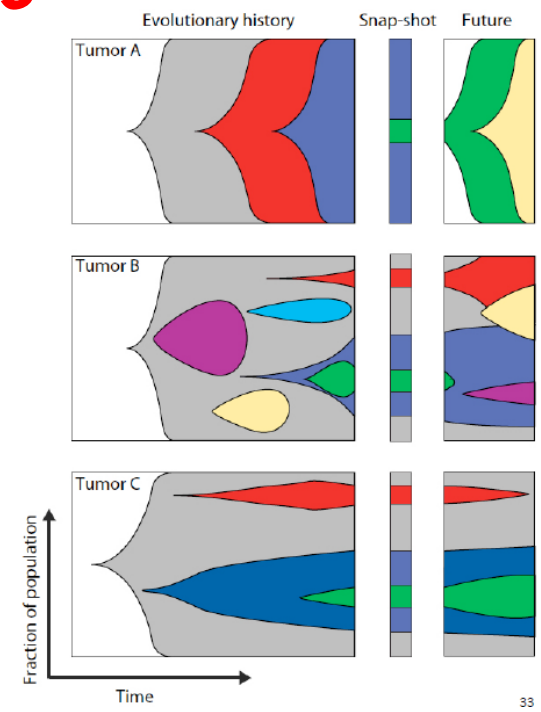

course of Gleevec treatment ⟶

    - But – the prognosis of CML appears now extremely positive.
        - Long-term results of Gleevec show sustained and durable responses with excellent estimated 5-year survival rates and minimal side effects.
        - For patients with Gleevec intolerance or progression, new tyrosine kinase inhibitors are providing excellent and durable responses.

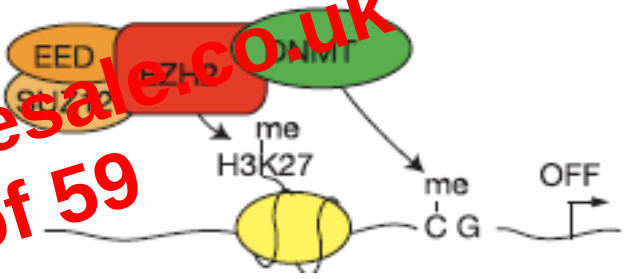
- Chromothripsis – relevance to cancer:
    - Multiple mutations at same time.
        - May disrupt several TS genes.
        - Could generate oncogenic gene fusions.
    - May be associated with more aggressive tumours.
        - In multiple myeloma and AML associated with poor prognosis.
        - In neuroblastoma, survival rates worse for tumours that have chromothripsis.
    - In sonic hedgehog subtype of medulloblastoma, chromothripsis associated with p53 mutations.
        - Also seen in AML.
        - Mechanistic link? G2/M checkpoint errors?



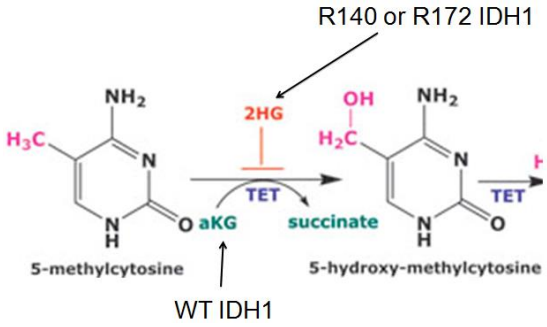
- Tumour heterogeneity and evolution:
    - Heterogeneity between different cancer types.
        - Different pathways, different driver genes.
        - Different mutational spectrum.
    - Heterogeneity within cancer types.
        - Different mutations in same driver genes.
        - Same pathway mutated, but at different points (e.g. RAS/BRAF in melanoma).
        - Different pathways, but result in same tumour
            - Can be important for therapy.
    - Heterogeneity within the same tumour/patient.
        - Tumour evolution
    - Linear evolutionary pattern (A):
        - Successive acquisition of advantageous mutations.
        - Relatively homogenous tumour.
        - This type of evolution has been observed in multiple myeloma and AML.
    - Branched evolutionary pattern (B and C):
        - Multiple distinct clones exist and co-evolve.
        - Heterogeneous tumour.
        - Potential for multiple subclonal driver events.
        - Dominant subclones can outcompete other clones.
        - Seen in many cancer types.
        - Implications for therapy.



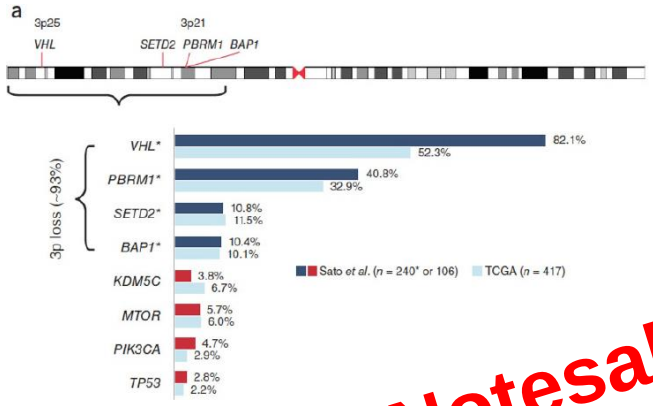
- Role of disrupted methylation in cancers:
    - Hypomethylation:
        - General hypomethylation in cancers.
        - Dnmt1 mutants get sarcomas and gut cancer.
        - Mechanism – gene expression and instability.
            - Dnmt3b mutant cells develop aneuploidy and chromosomal breaks.
    - Hypermethylation:
        - Sometimes 'replaces' LOH, i.e. inactivation of second copy of a tumour suppressor gene.
        - Tumour suppressor genes.
            - More than half TS genes have been found hypermethylated.
            - Some (RASSF1A) almost always inactivated this way.
- Methylation of CpG islands suppresses gene expression:
    - CpG islands are:
        - At least 200 bp.
        - Greater than 50% GC.
        - Observed-to-expected CpG ratio >60%.
            - Observed-to-expected CpG ratio = ((Num of CpG/(Num of C × Num of G)) × Total number of nucleotides in the sequence).
- What causes a methylator phenotypes:
    - Defects in the methylation machinery.
        - DNMTs (DNA-methyltransferases).
        - Regulators of methylation machinery.
        - Polycomb etc.
    - H3K27me3 is a repressive mark.



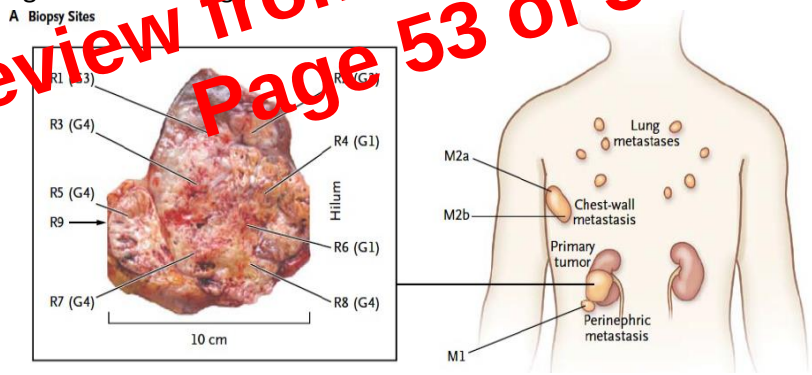
- What causes a methylator phenotypes?
    - Epigenetics DNA methylation – DNMT3A.
    - Epigenetics DNA hydroxymethylation – TET2.
    - Metabolism – IDH1 and IDH2.
- What causes a methylator phenotypes – mutation of methylators.
    - AML (adults) often have dominant negative DNMT3A mutation.
        - Maybe not so frequent.
- What causes a methylator phenotypes – mutation of demethylators.
    - Tet catalyses first step in demethylation.
    - Mutation of IDH1 or IDH2 actually changes activity so leads to decreased α-KG increased synthesis of 2-HG which inhibits activity of TET family of DNA hydroxylases.
    - Tet families remove methyl groups from cytosine – involved in demethylation.
    - Mutations events are in changes of the machinery that takes methyl group off – demethylation machinery broken.
    - α-keto glutamate is a donor which is inhibited.



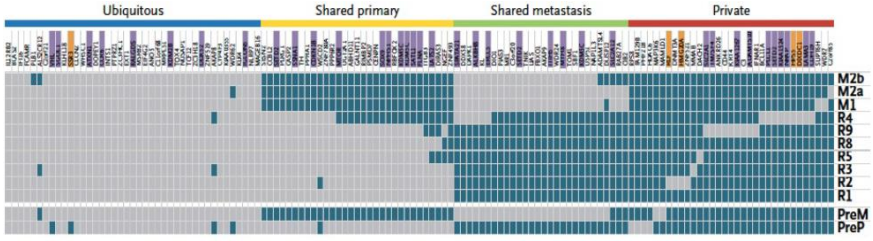
- Clear cell renal carcinoma (ccRCC):
    - Represents 75% of kidney cancers.
        - Affects 300,000 people worldwide every year.
        - 100,000 deaths annually.
    - Sporadic and heritable forms.
        - Most inherited cases have mutations in VHL gene (von Hippel-Lindau disease gene).
        - VHL mutated or hypermethylated in >90% of sporadic ccRCC.
            - Loss of chromosome 3p (contains VHL) almost always occurs as well.
        - VHL mutation is a very early event.
    - Tumours slow growing.
    - Used to study genomic architecture and evolution of tumours.
- Genomic mutations in ccRCC:



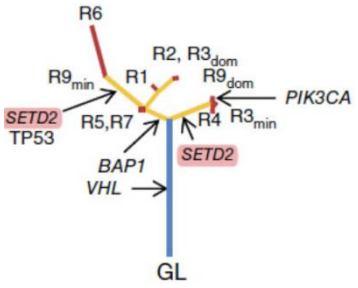
    - Note that loss of 3p removes one copy of 4 key TS genes.
- Identifying tumour heterogeneity in ccRCC:



- Patterns of mutations in one tumour:



    - Dark boxes represent non-synonymous mutations.
- The relationship between the mutations in the different sites can be represented as a phylogenetic tree.
- Different tumours follow different (but related) evolutionary:



- Homeopathy costs NHS £4,000,000/year:
    - Survey of more than 1,000 clinical staff – 55 per cent want to see an immediate ban on funding homoeopathy and herbal medicine.
- Cancer prevention?
    - Basic premise of many:
        - Immune system, inflammation, glycolytic metabolism.
        - Easy for viruses, e.g. HPV in cervical cancer.
    - Evidence – some:
        - Cancer getting more common in the West – therefore diet?
        - 1/3 of cancers 'avoidable'.
        - Green tea, curcumin.
            - Three meals a day – so regular treatment!
    - Do herbal/folk medicines have any value?
    - Is there a tree that produces the cancer wonderdrug?
- Alternative therapy and prevention – aspirin (an NSAID):
    - 400 BC – Hippocrates prescribes the bark and leaves of the willow tree (rich in a substance called salicin) to relieve pain and fever.
    - In 1828, Johann Buchner, professor of pharmacy at the University of Munich, isolated a tiny amount of bitter tasting yellow, needle-like crystals, which he called salicin.
    - 75mg or more per day vs control – used for the prevention of vascular events in the UK.
    - 27% less likely than controls to die from cancer.
    - Reduced risk of death from adenocarcinoma with metastasis at initial diagnosis by 31%.
    - Reduced the risk of metastasis in patients without initial metastasis by 55% (colorectal cancer by 74%).

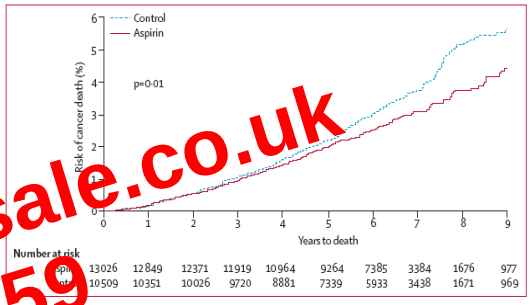
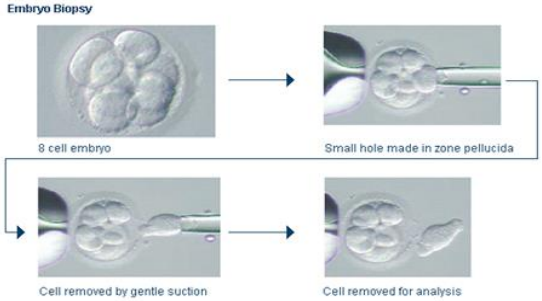


Figure: Effect of allocation to aspirin versus control on risk of death due to cancer during the trial treatment periods in a pooled analysis of the 23 535 patients in seven trials[57-21,324]

- Aspirin – mechanism:
    - Unclear – could be via increasing apoptosis of cancer cells.
    - COX-2 inhibition, since many colorectal cancers aberrantly express COX-2.
    - Antiinflammatory – some immune cells promote cancers.
- Cancer free babies born:
    - First used for the severe, inherited cancer predisposition Li Fraumeni Syndrome, resulting in the birth in the USA in 2001 of a child that had been selected to be free of the mutant gene that was carried by its parents.
- Preimplantation genetic diagnosis (PGD):
    - Cancer syndromes:
        - UK – BRCA1, 2, Rb, Gorlins, HNPPC, etc.
        - USA – BRCA1, APC, PTCH, P53, RB1, VHL, many others.
    - ART – intracytoplasmic sperm injection.



- Antibody therapy of cancer:
    - Antibody-based therapy for cancer has become established over the past 15 years and is now one of the most successful and important strategies for treating patients with haematological malignancies and solid tumours.
    - Evidence from clinical trials of antibodies in cancer patients has revealed the importance of iterative approaches for the selection of antigen targets and optimal antibodies.
    - The killing of tumour cells using monoclonal antibodies (mAbs) can result from direct action of the antibody (through receptor blockade, for example), immune-mediated cell killing mechanisms, payload delivery, and specific effects of an antibody on the tumour vasculature and stroma.
    - Tumour antigens that have been successfully targeted include epidermal growth factor receptor (EGFR), ERBB2, vascular endothelial growth factor (VEGF), cytotoxic T lymphocyte-associated antigen 4 (CTLA4), CD20, CD30 and CD52.
    - Serological, genomic, proteomic and bioinformatic databases have also been used to identify antigens and receptors that are overexpressed in tumour cell populations or that are linked to gene mutations identified as driving cancer cell proliferation, including EGFRvIII, MET, CTLA4 and fibroblast activation protein (FAP).
    - The successful development of candidate mAbs for the clinic involves a complex process of scientific and preclinical evaluations that include identification of the physical and chemical properties of the antibody; the detailed specificity analysis of antigen expression; the study of the immune effector functions and signalling pathway effects of the antibody; the analysis of in vivo antibody localization and distribution in transplanted or syngeneic tumour systems; and the observation of the in vivotherapeutic activity of the antibody.
    - A major objective for the clinical evaluation of mAbs has been determining the toxicity and therapeutic efficacy of the antibody alone or as a delivery system for radioisotopes or other toxic agents. It is also crucial to assess its in vivo specificity by determining its biodistribution in patients and to assess the ratio of antibody uptake in the tumour versus normal tissues.
    - Twelve antibodies have received approval from the US Food and Drug Administration for the treatment of various solid tumours and haematological malignancies, and a large number of additional therapeutic antibodies are currently being tested in early stage and late-stage clinical trials.